

L'inhibition de l'EGFR dans les CBNPC

Une thérapeutique personnalisée en pleine ... mutation

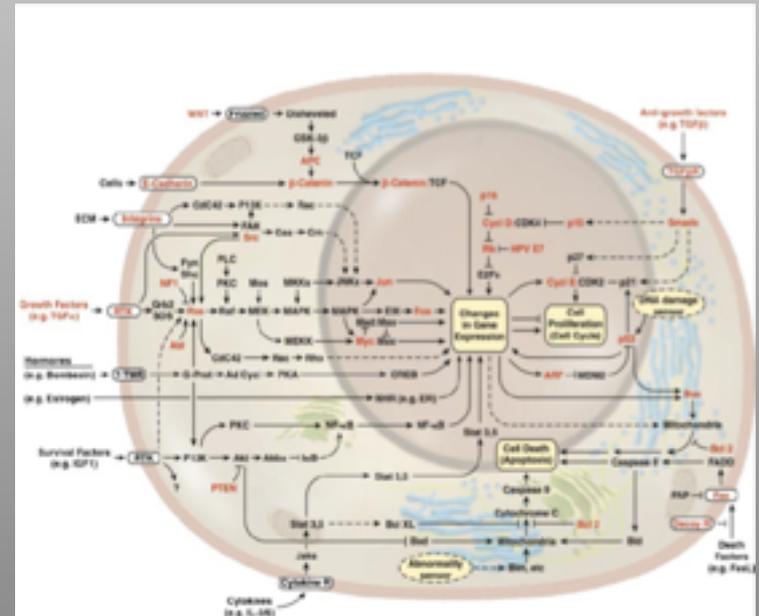
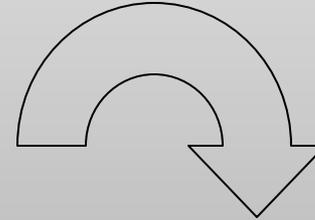
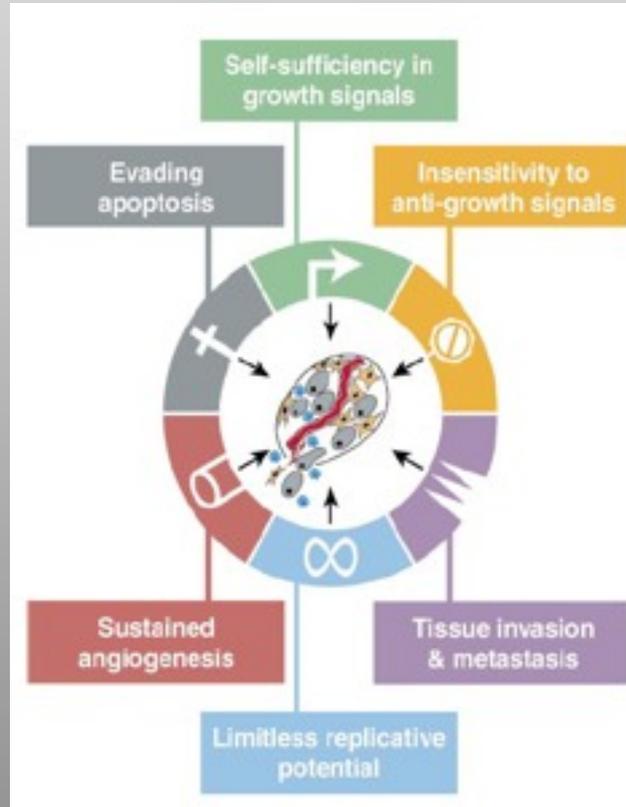
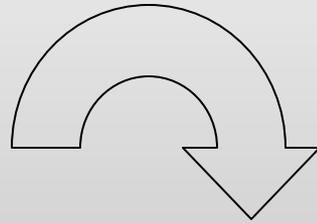
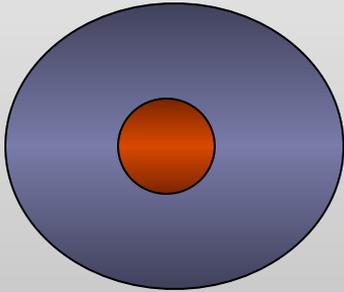
Michèle Beau-Faller

Laboratoire de Biochimie et de Biologie Moléculaire, CHU de Strasbourg

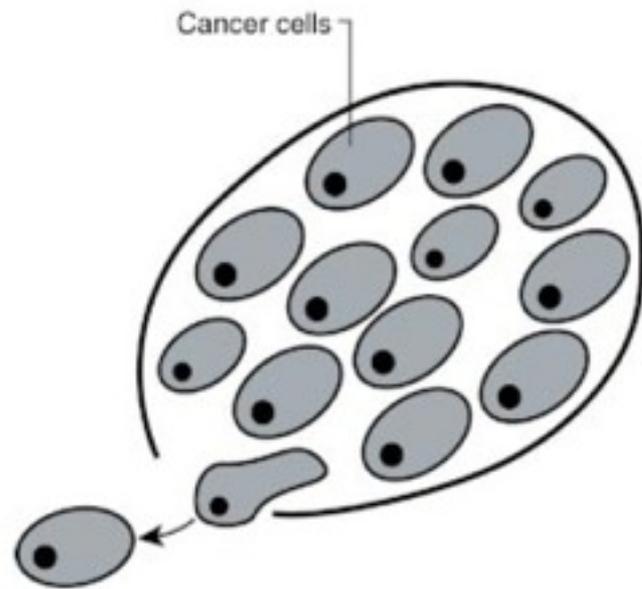
EA 4438 Groupe « Marqueurs moléculaires de progression tumorale

et de sensibilité aux drogues anticancéreuses" - UdS

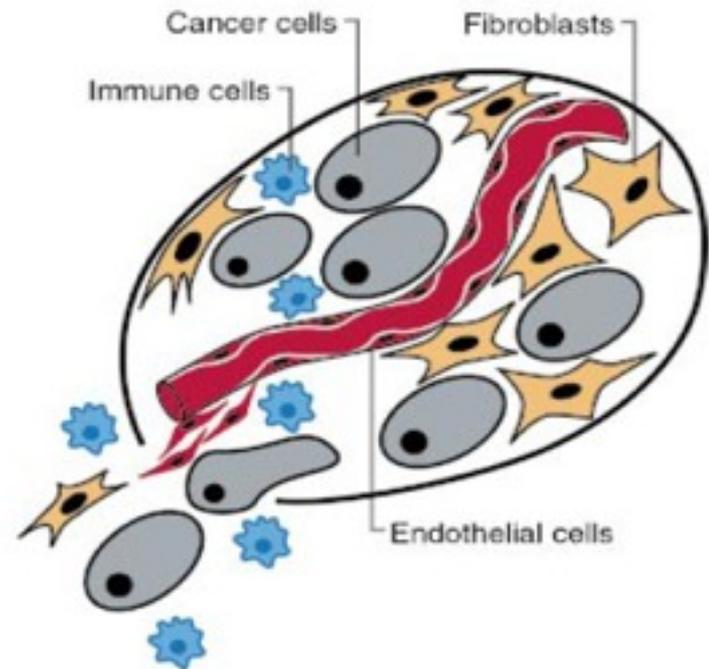
Fondements biologiques



The Reductionist View



A Heterotypic Cell Biology



La saga de l'EGFR ...

ADN



ARN



Protéine

Les différentes classes de récepteurs à activité TK

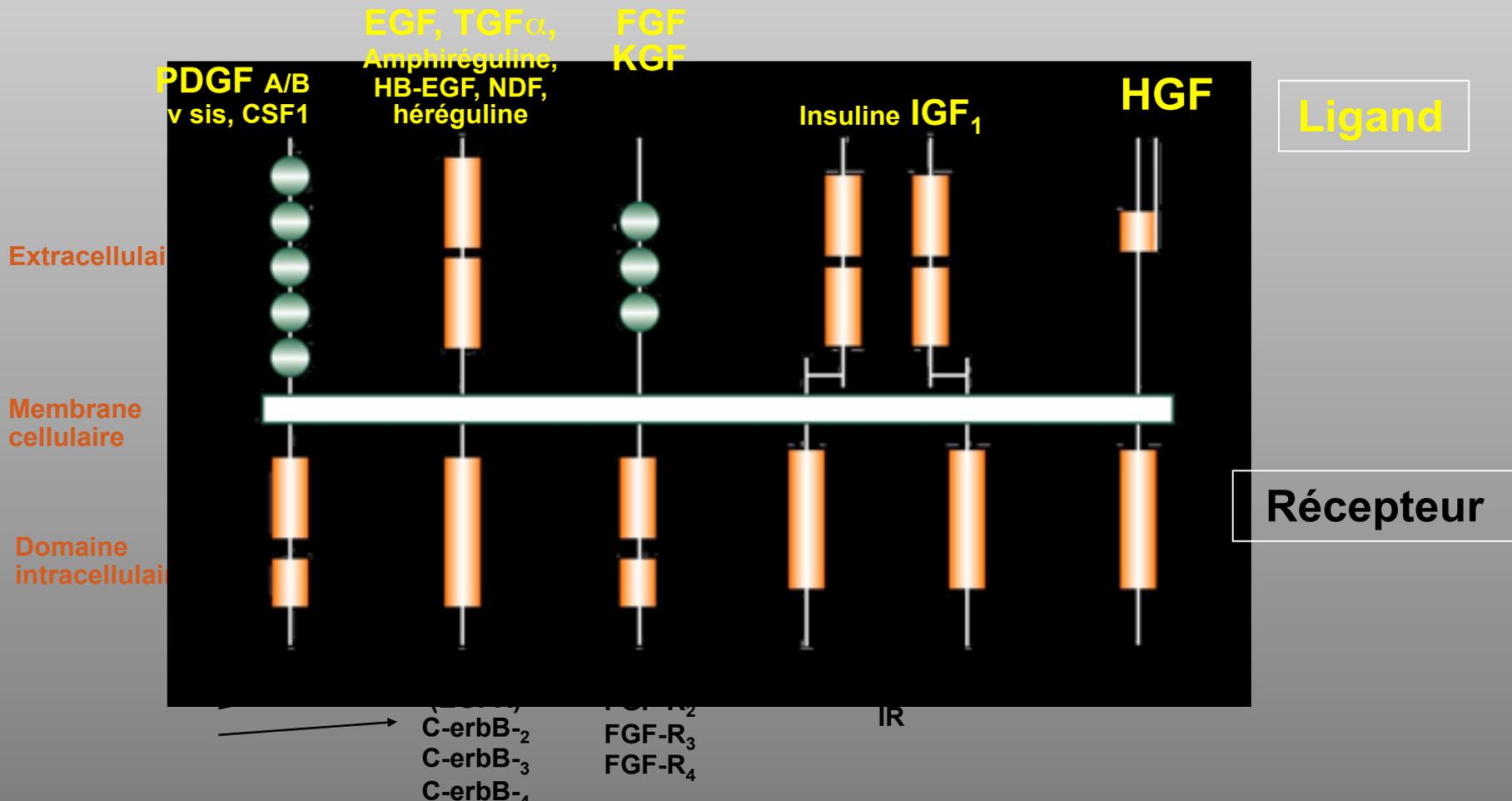
III
PDGF-R

I
EGF-R

IV
FGF-R

II
IGF-R

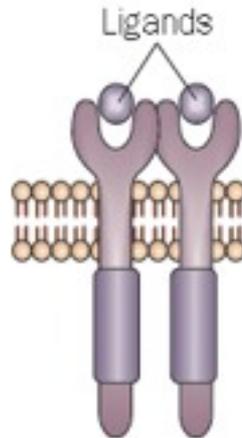
V
MET



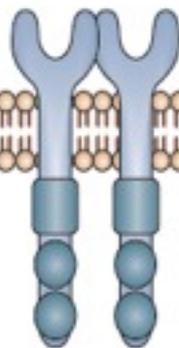
Ce qui est connu

a EGFR-EGFR HER2-HER2 EGFR-HER2 HER2-HER3 HER2-HER4

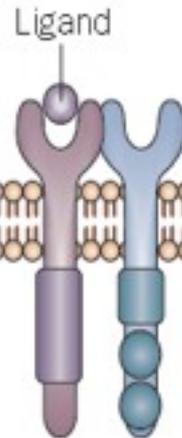
EGF
TGF- α
Amphiregulin
HB-EGF
Betacellulin
Epiregulin



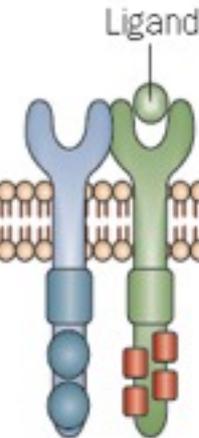
HER2-HER2



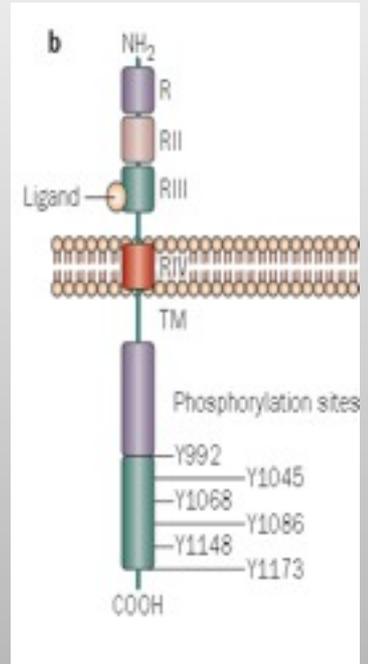
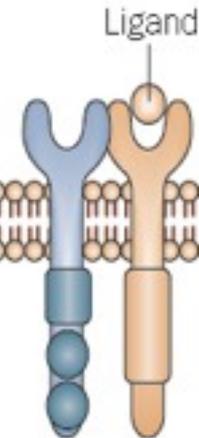
EGFR-HER2



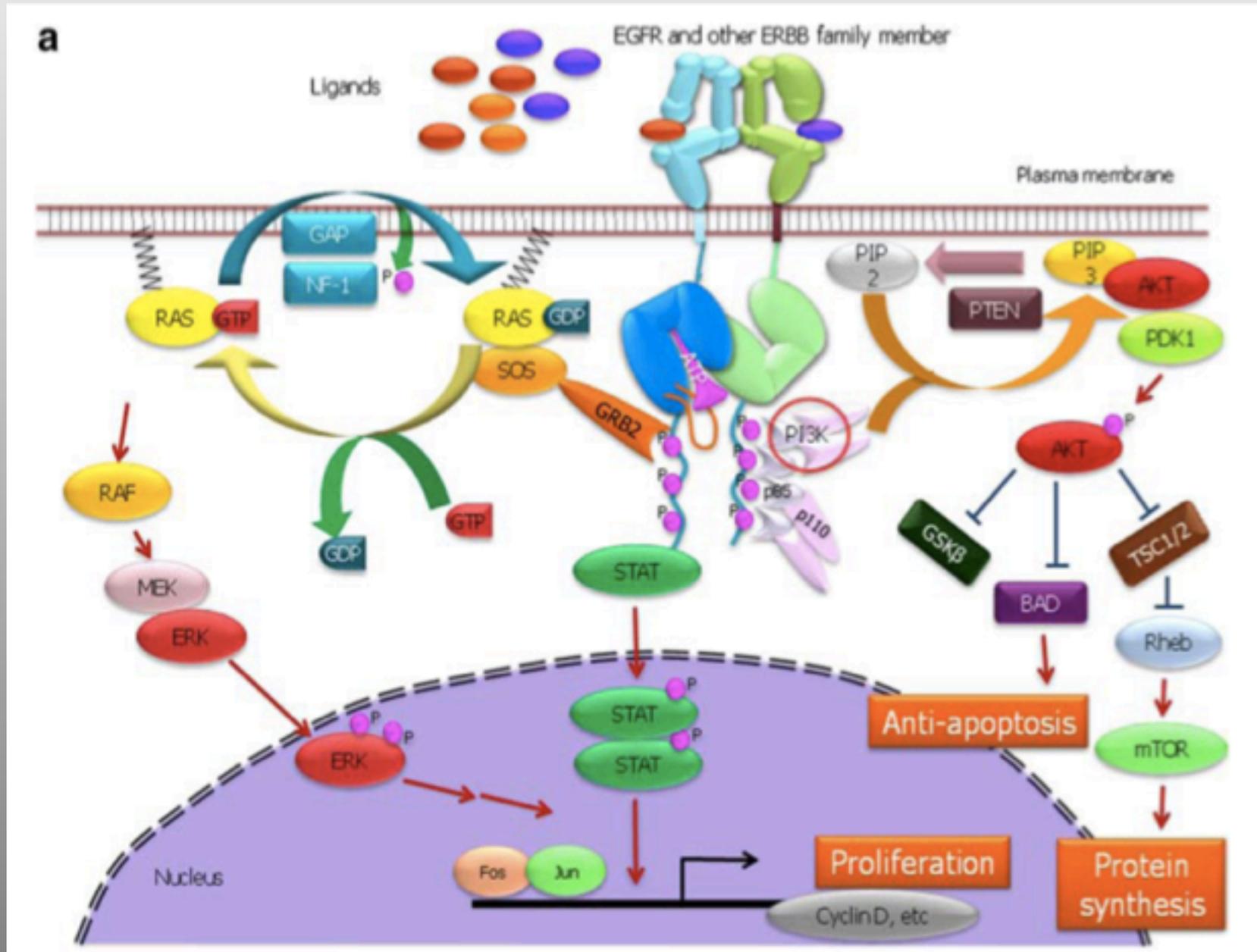
HER2-HER3



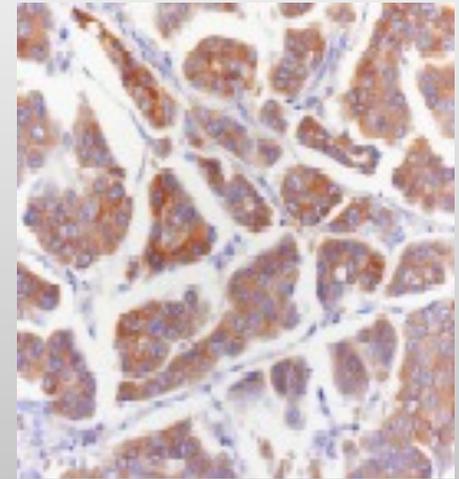
HER2-HER4



Voies de signalisation



Surexpression EGFR



- Fréquente
 - > 50% CBNPC
 - E (55-100%) > ADC (35-60%) > GC (20-60%)
- Valeur pronostique: controversée Kakamura H, Thorax 2006
- Pas de valeur prédictive évidente pour les EGFR-TKI

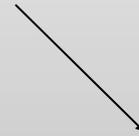
(IDEAL, INTACT, TALENT, TRIBUTE, et BR21, ISEL, SATURN)

- Aspects techniques
- Conservation des blocs

- Valeur prédictive pour mAb cetuximab

(FLEX) Pirker R et al, JCO 2008

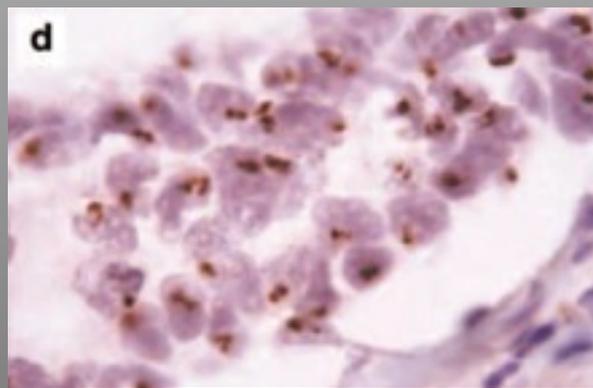
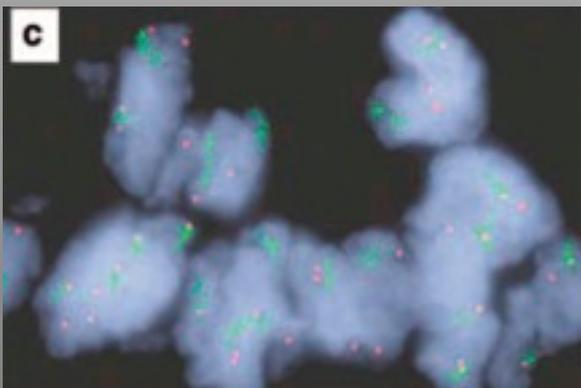
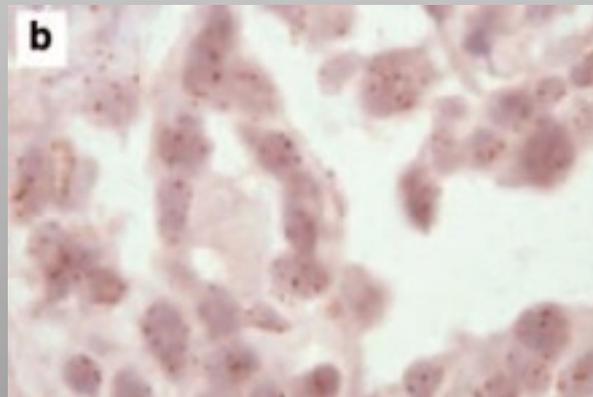
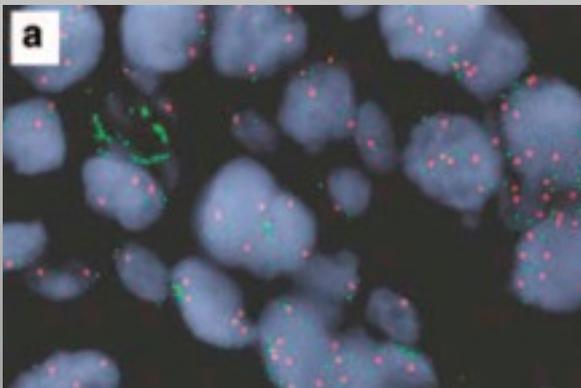
Nombre de copies d'EGFR



FISH

CISH

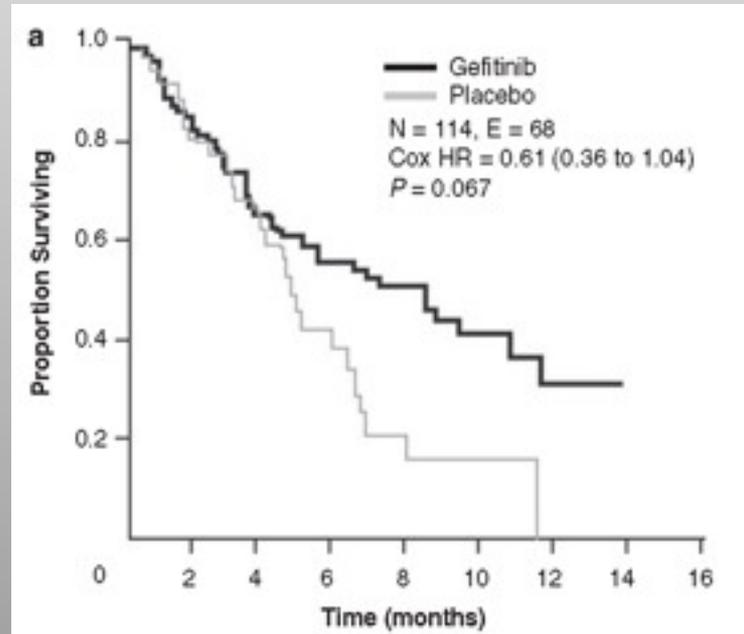
QPCR



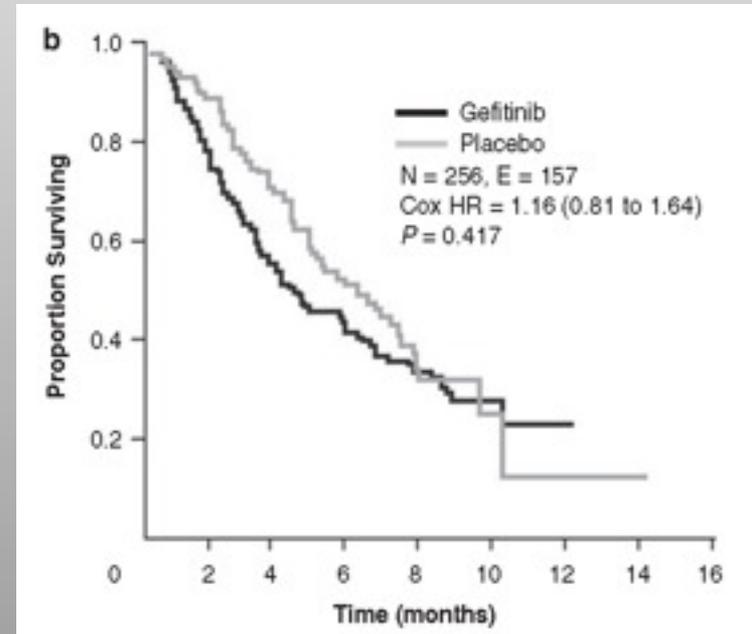
polysomie
30-50%

amplification
10%

Etude ISEL



FISH EGFR +



FISH EGFR -

Co-expression EGFR/HER-2

- Immuno-histochimie (Hercep Test)
 - 18-33% CBNPC
 - ADC > LC > E
 - Chimiorésistance
 - Co-expression: facteur de mauvais pronostic
- FISH: le double gain est un facteur prédictif et pronostic

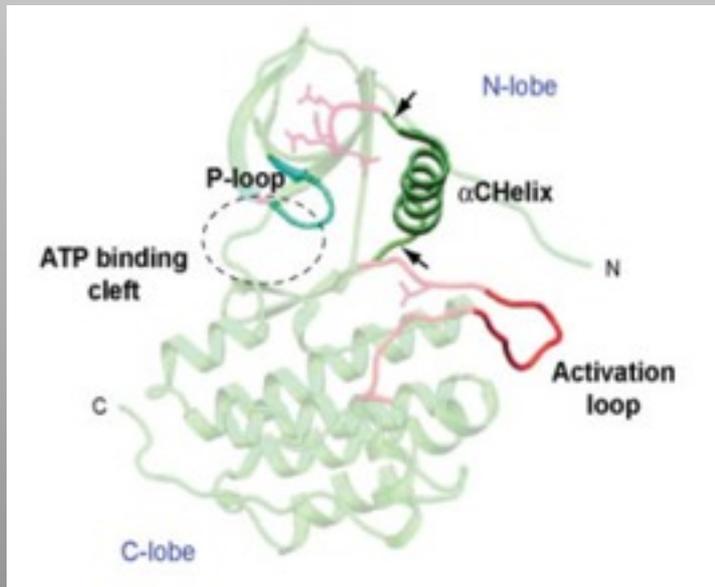
Table 1 Positive HER2 status as assessed by FISH is associated with enhanced prognosis in patients with EGFR-positive NSCLC

	<i>EGFR-positive/ HER2-positive (n = 13)</i>	<i>EGFR-positive/ HER2-negative (n = 19)</i>	<i>EGFR-negative/ HER2-positive (n = 10)</i>	<i>EGFR-negative/ HER2-negative (n = 59)</i>	<i>Any positive (n = 42)^a</i>	<i>Any negative (n = 88)^b</i>
ORR, %	54	21	10	2	29	7
DCR, %	77	58	30	25	58	33
Median TTP, mo	9.8	5.3	2.3	2.6	6.0	2.6
Median OS, mo	20.8	5.8	6.0	7.3	13.8	8.3

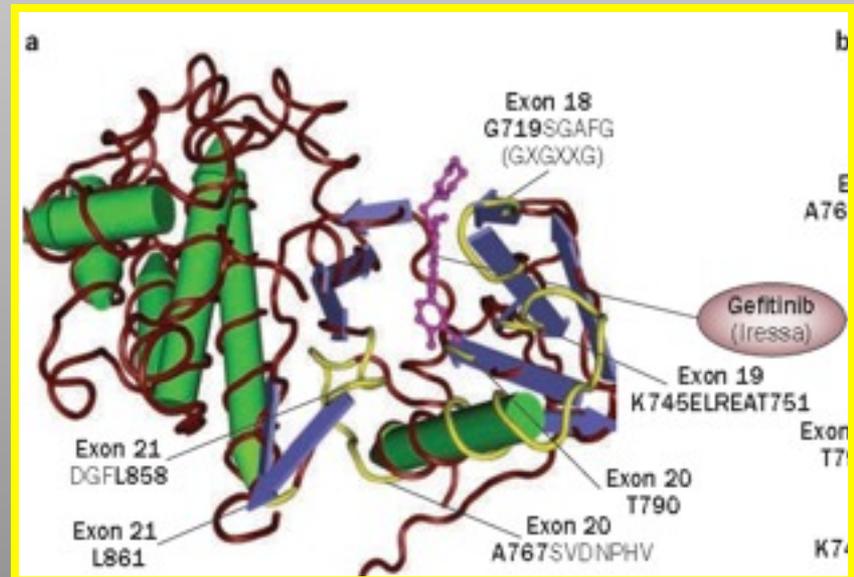
Mutations somatiques d'EGFR

- Mutations activatrices et prédictives de sensibilité aux EGFR TKI identifiées en 2004 : exons 18 à 21

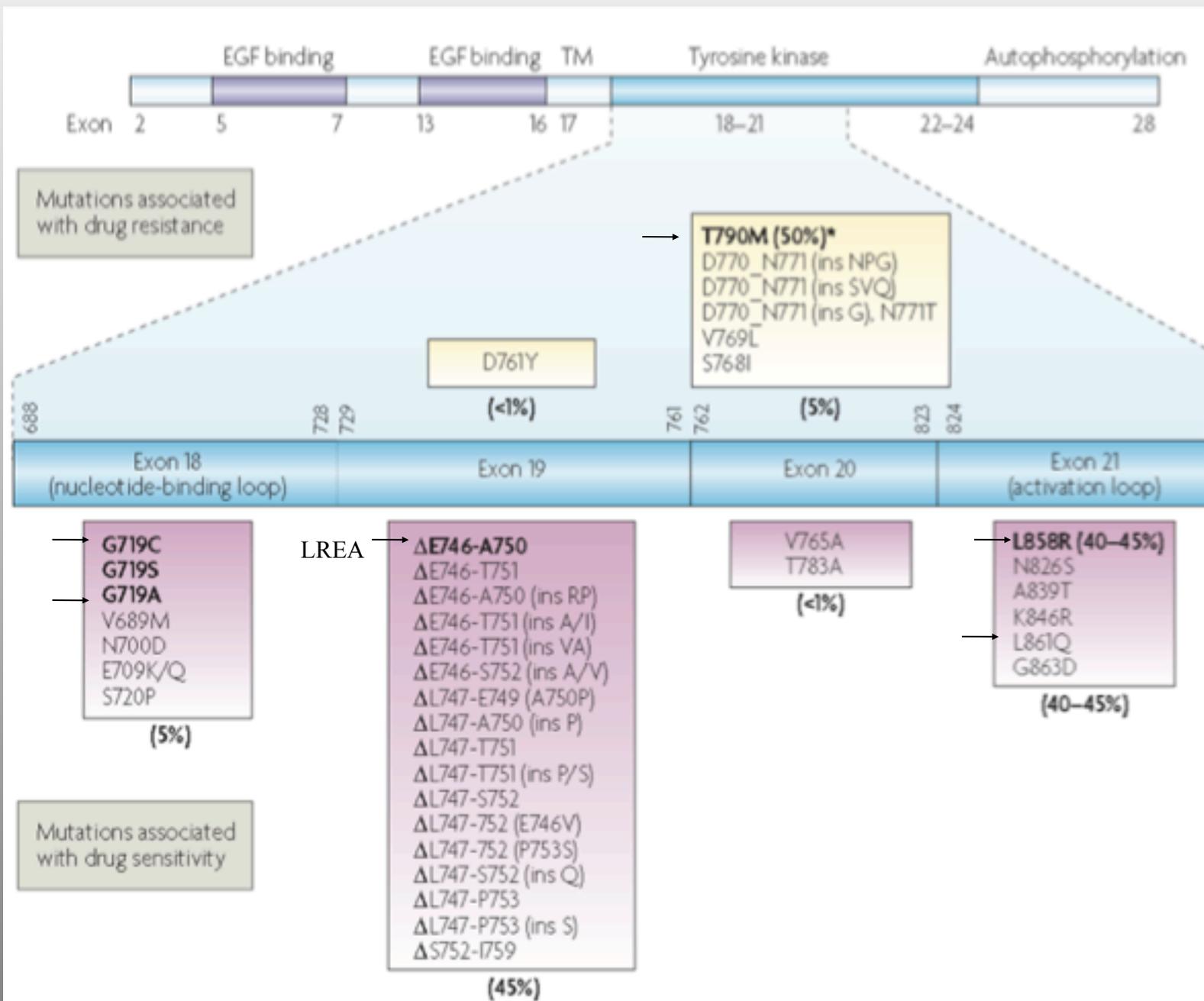
Lynch TJ NEJM 2004; Paez JG Science 2004; Pao W JCO 2005



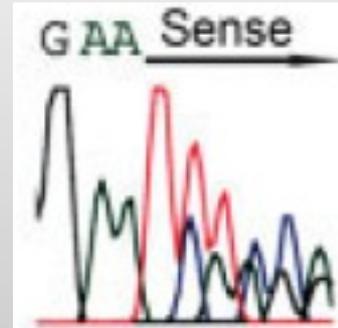
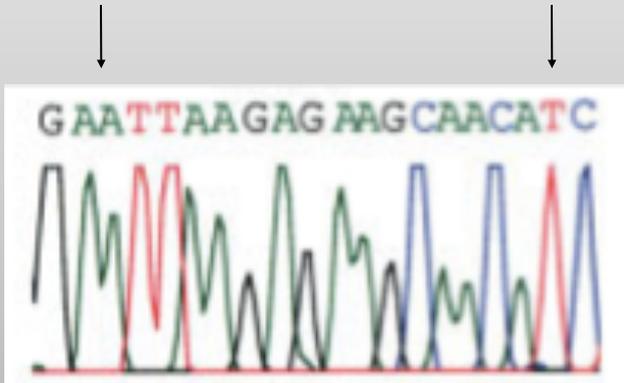
Shigematsu IJC 2006



Linardou H, Nature 2009



Exon 19



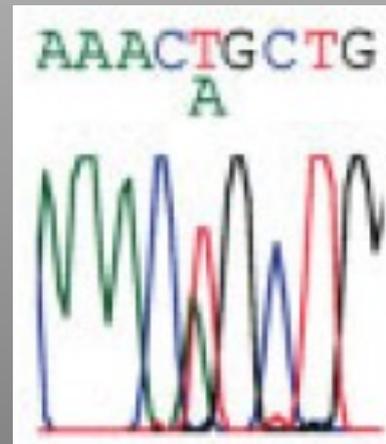
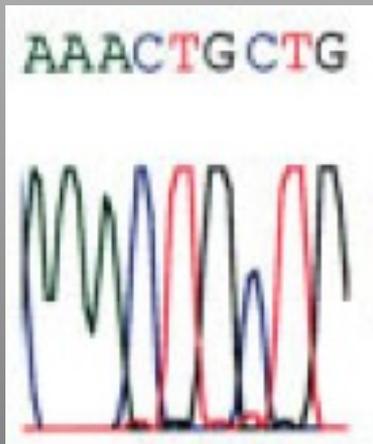
Hétérozygote



Homozygote

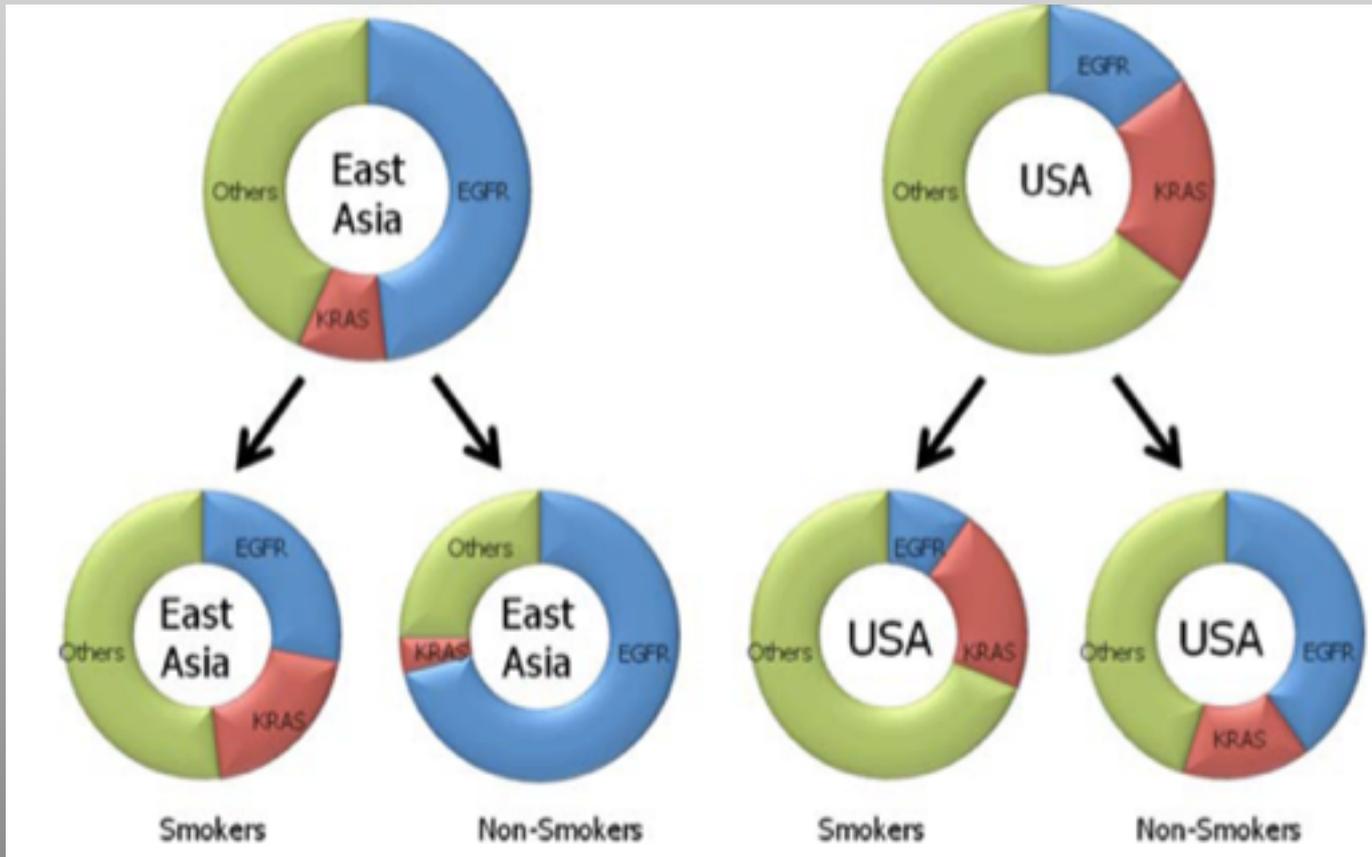
DelL747-L751

Exon 21



L861Q

Indicence des mutations EGFR



Mutations d'EGFR et données cliniques

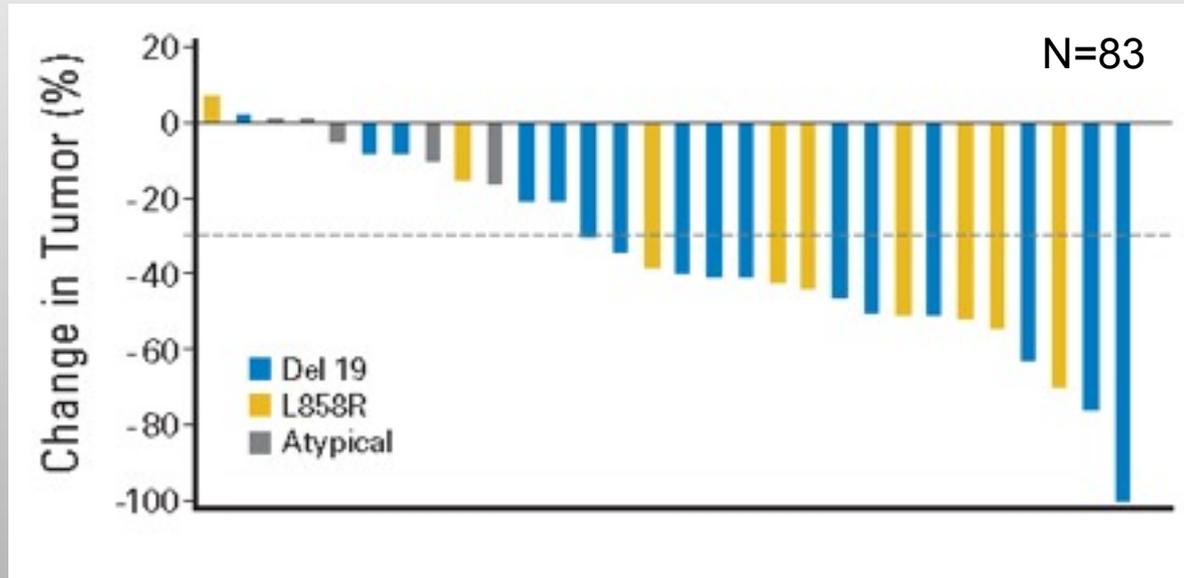
- ADC, 5-20 % des Caucasiens, 20-40 % des Asiatiques, sexe féminin, non-fumeur
- Corrélation au statut tabagique
 - Forte corrélation entre le statut non-fumeur et la présence de mutations
 - Pour chaque PA, la probabilité d'avoir une mutation d'*EGFR* diminuée de 4%
Sequist L CCR 2006

	<i>Total, %</i>	<i>Non-east Asian, %</i>	<i>East Asian, %</i>
All subgroups	19	10	30
Smokers	11	4	17
Nonsmokers	54	35	60
Adenocarcinoma	42	16	49
Non-adenocarcinoma	3	1	4
Male	16	1	22
Female	46	20	58

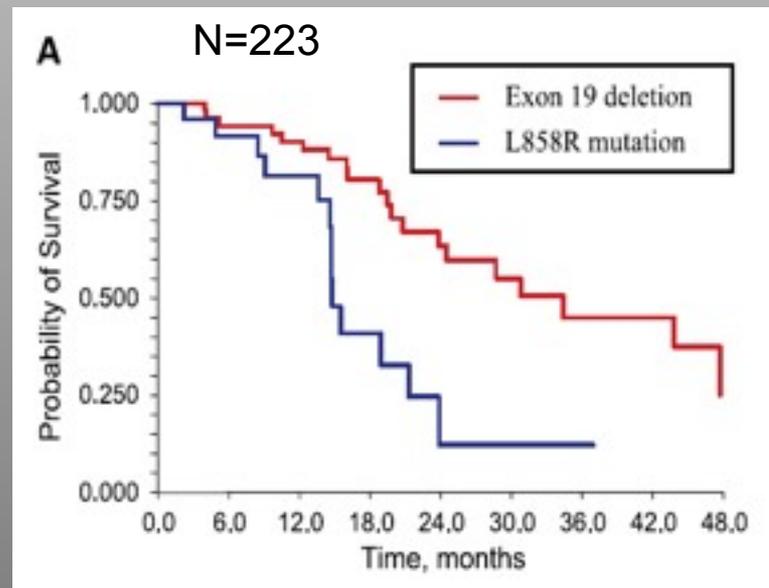
Jänne PA CCR 2006

- Génotype EGFR sélectionnerait mieux les patients répondeurs à un EGFR-TKI en 1ère ligne que les critères cliniques

Valeur prédictive



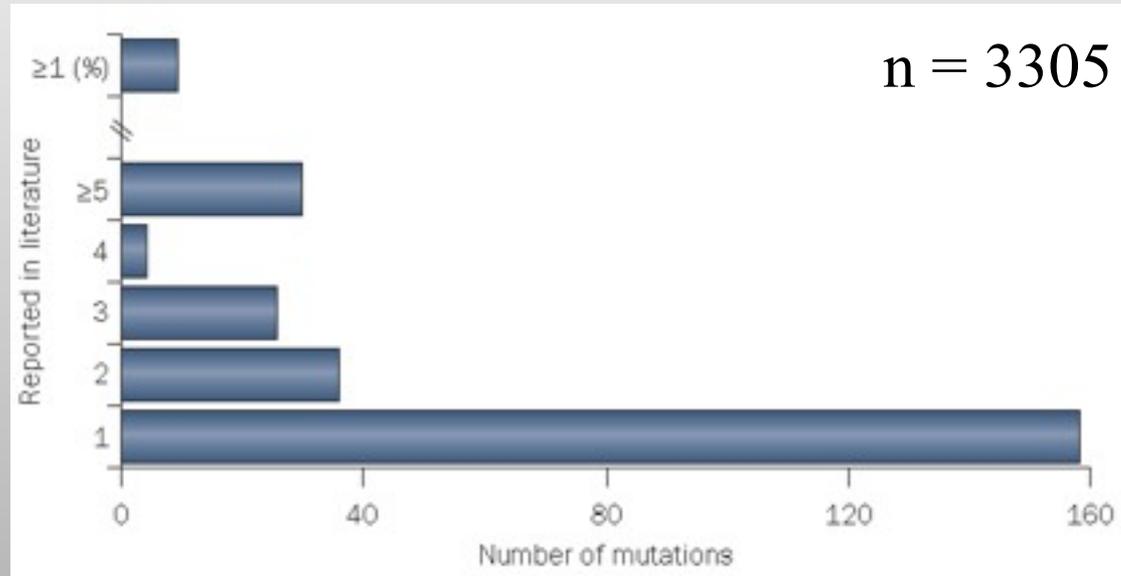
Sequist LV, JCO 2008



Jackman DM CCR 2009

EGFR: les nouveautés

Incidence des mutations d'EGFR

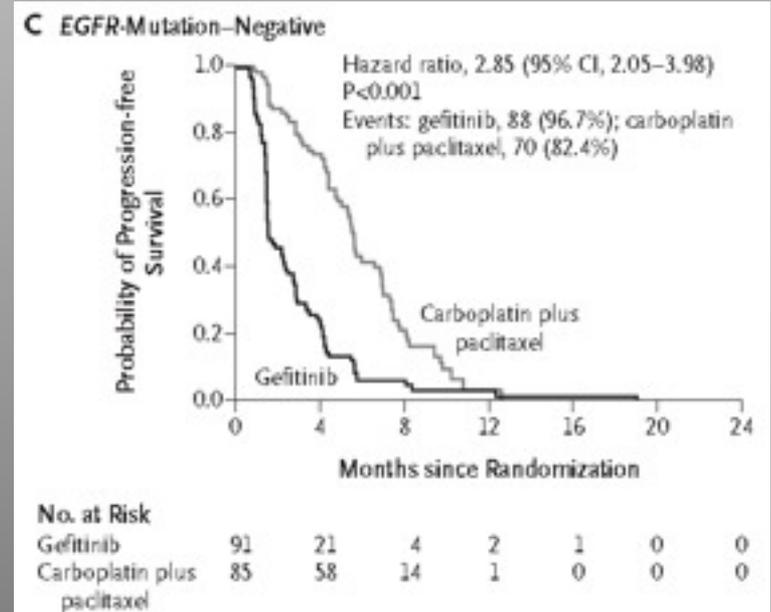
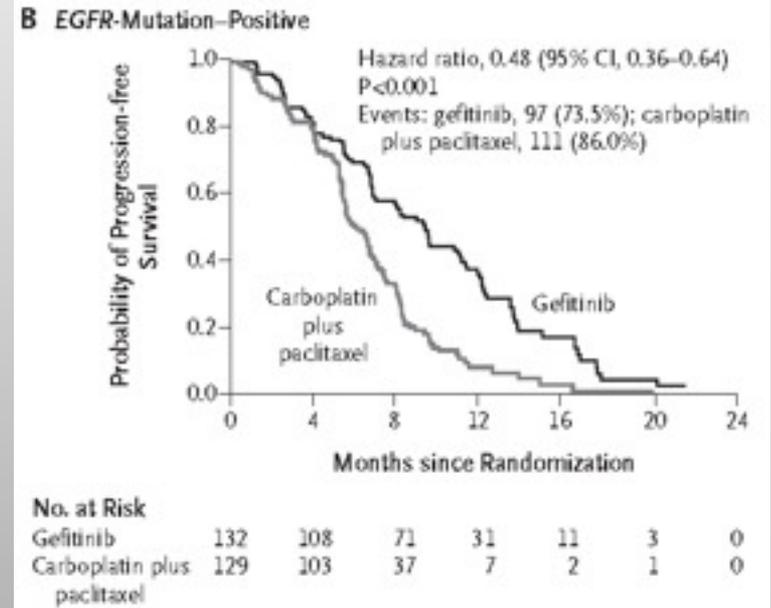
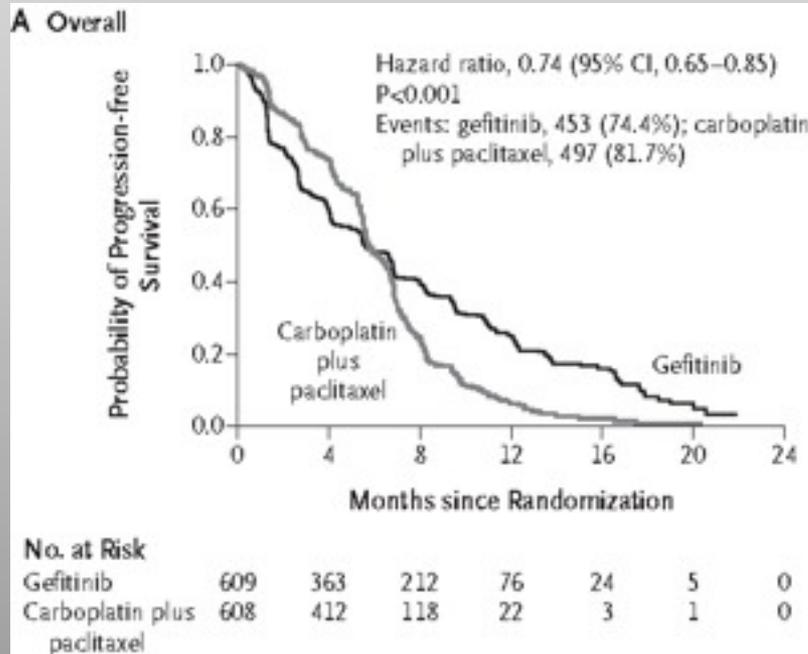


Réponse avec une monothérapie EGFR TKI

Response	Wild-type/ mutant (%) n=975/561	Ex19del (%) n=442	L858 (%) n=232	T790M (%) ^a n=33	Reported incidence ≥1 (%) n=59	Reported incidence <5 (%) n=106	Non-Ex19del mutations in exon 19 (%) n=16	Non-L858 mutations in exon 21 (%) n=37
CR and/or PR	9.8/67.0	76.9	63.8	0.0	57.6	66.0	46.7	64.9
SD	31.7/18.6	10.2	13.8	3.0	10.2	11.3	13.3	18.9
PD	54.0/9.6	4.5	6.9	97.0	25.4	17.9	33.3	16.2

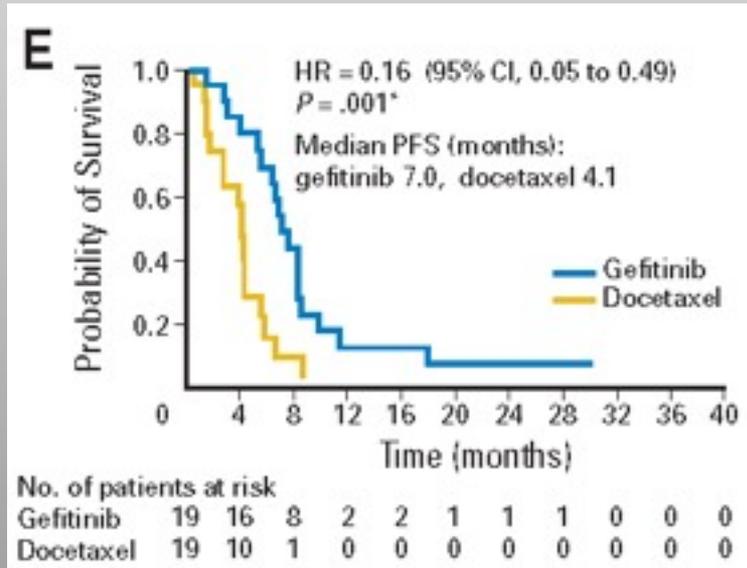
^aPatients with single T790M mutation. Abbreviations: CR complete response; PD, progressive disease; PR, partial response; SD, stable disease; TKI, tyrosine kinase inhibitor.

IPASS



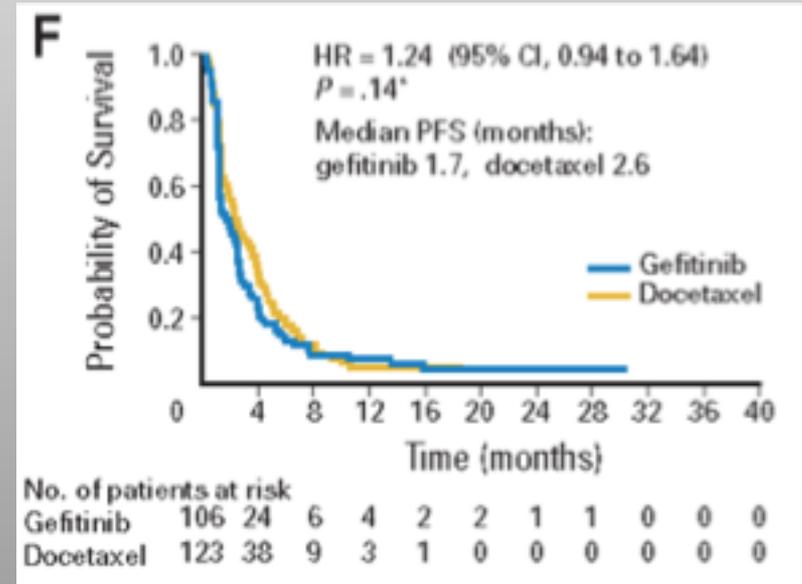
INTEREST-Bio

PFS



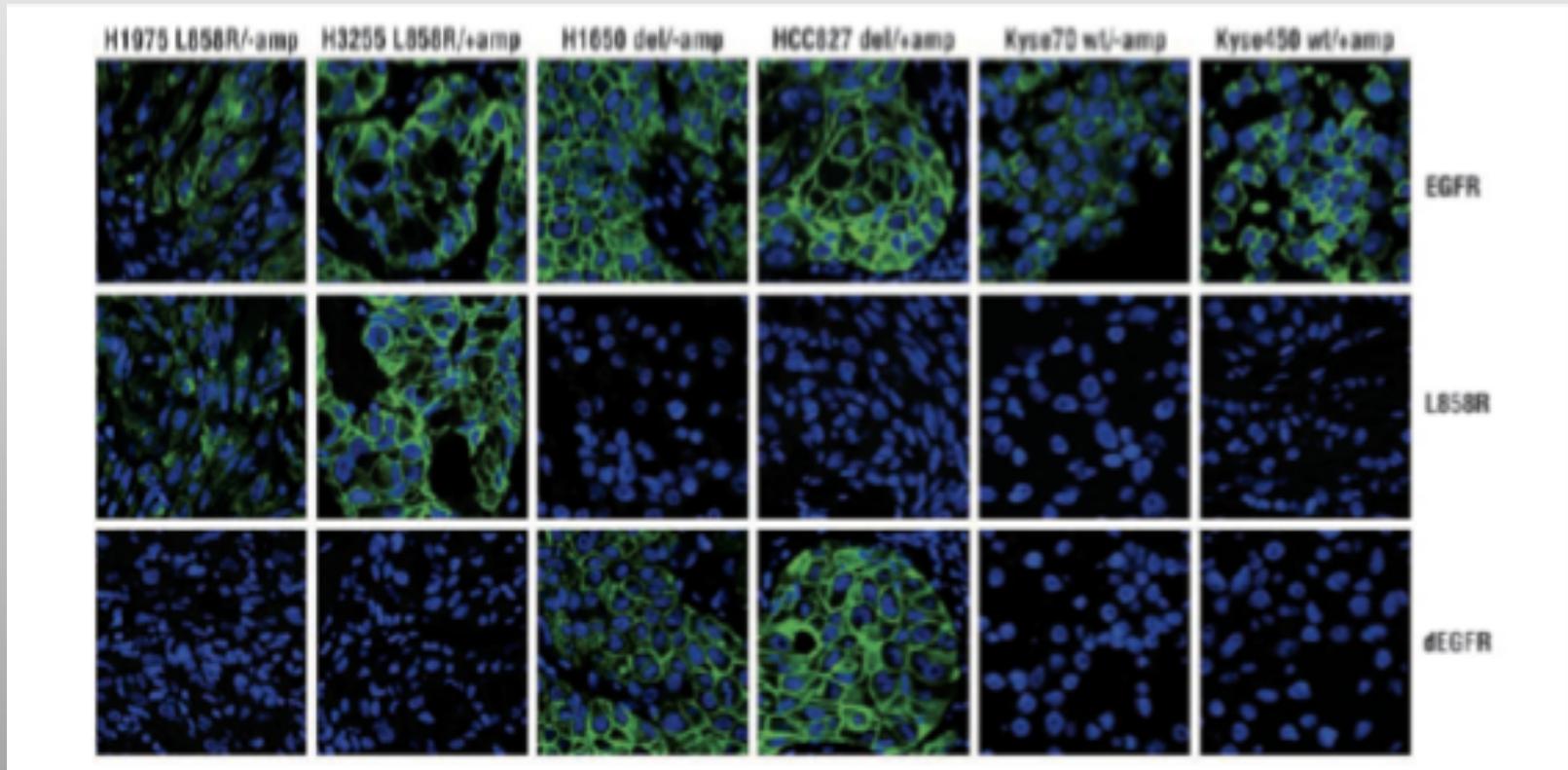
EGFR muté

PFS



EGFR non muté

Futur: IHC ?



Sensibilité :90- 67%; spécificité 99%

Yu J CCR 2009,
Brevet M J Mol Diag 2010

Mais

- Mutations ciblées donc non exhaustives
- Problèmes de cut off (sensibilité 95% (1+) à 76% (2+))

EGFR: les problèmes

Résistances aux EGFR-TKI

- Résistance primaire

- Ex19del : 4.2% et L8958R: 6.8% de résistance! Murray S JTO 2008
- Insertions exon 20: 2% des patients
- Mutations K-Ras:
 - 20% des CBNPC
 - ADC, fumeurs
 - Exclusivité avec EGFR
 - Activation constitutive de la voie EGFR
 - Peu ou pas de réponse aux EGFR-TKI (méta-analyse) Linardou H

Lancet Oncol 2008

K-Ras

- protéine clé de la transduction du signal dans grand nombre de voies de signalisation
- mutations codon 12, 13, 61: perte de l'activité GTPase: activation constitutive

- clinique:

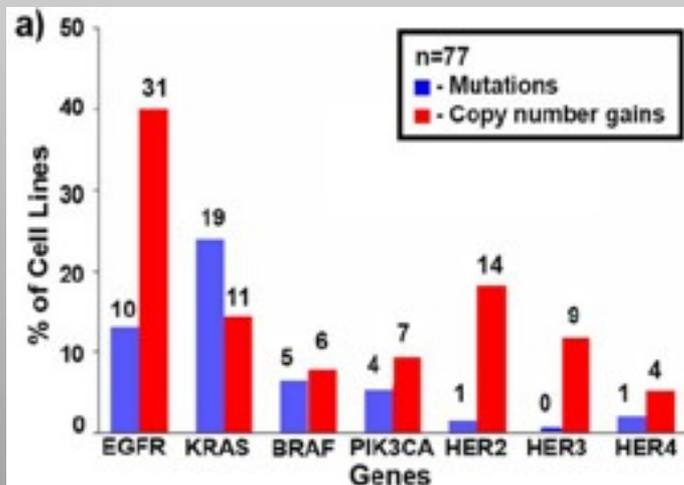
- 15-30% des ADC
- fumeur

MAIS 15% de non-fumeur!

- valeur pronostique controversée
- valeur prédictive négative pour EGFR-TKI
- pas de valeur prédictive démontrée pour les chimiothérapies cytotoxiques

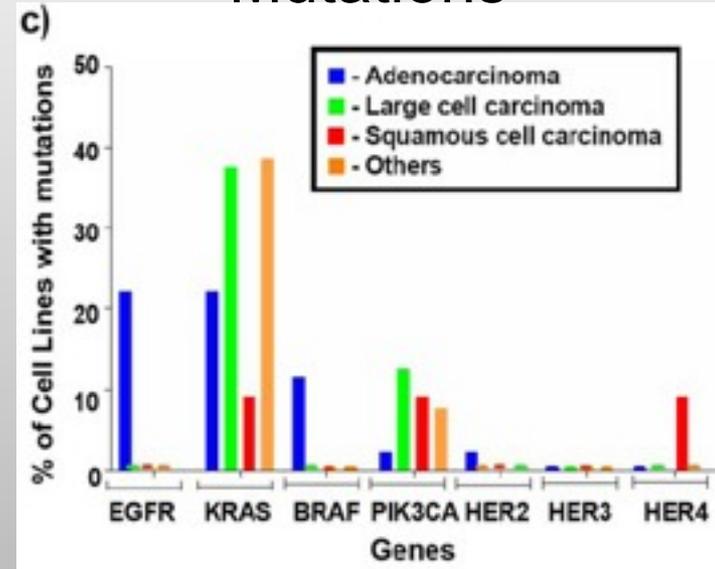
MASI

Mutant Allelic Specific Imbalance

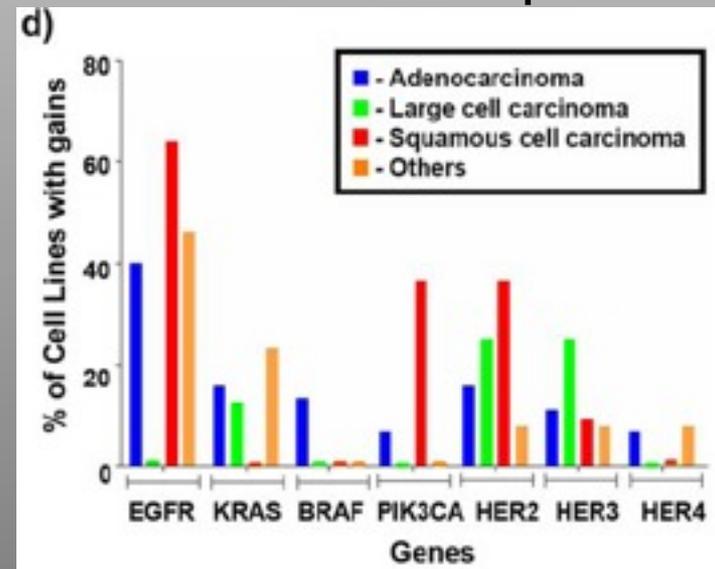


Dans 39 lignées de CBNPC

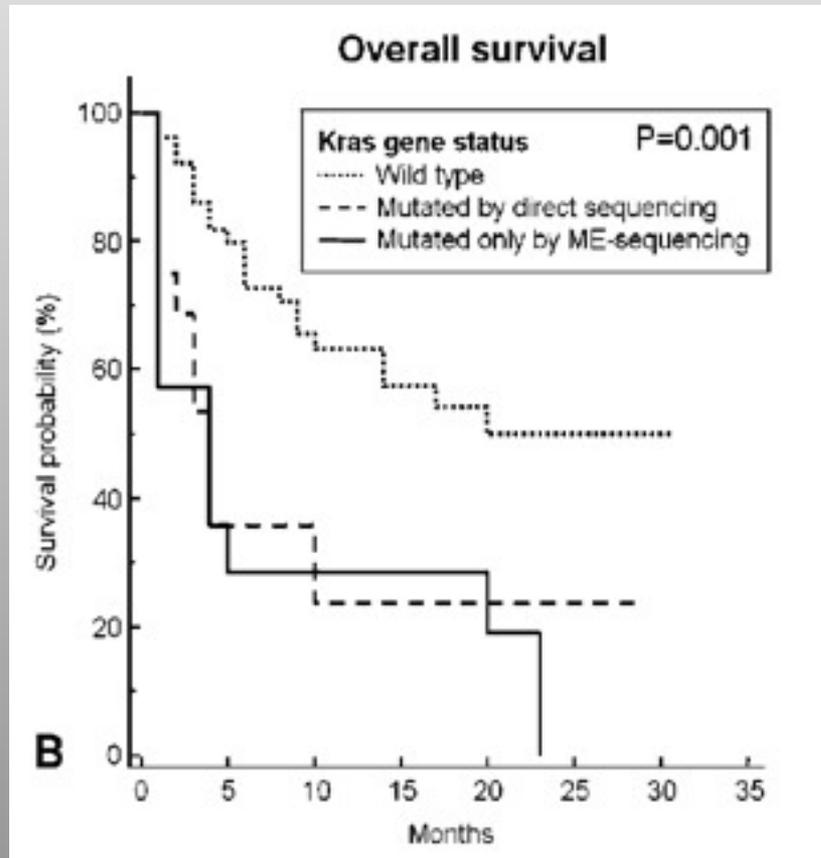
Mutations



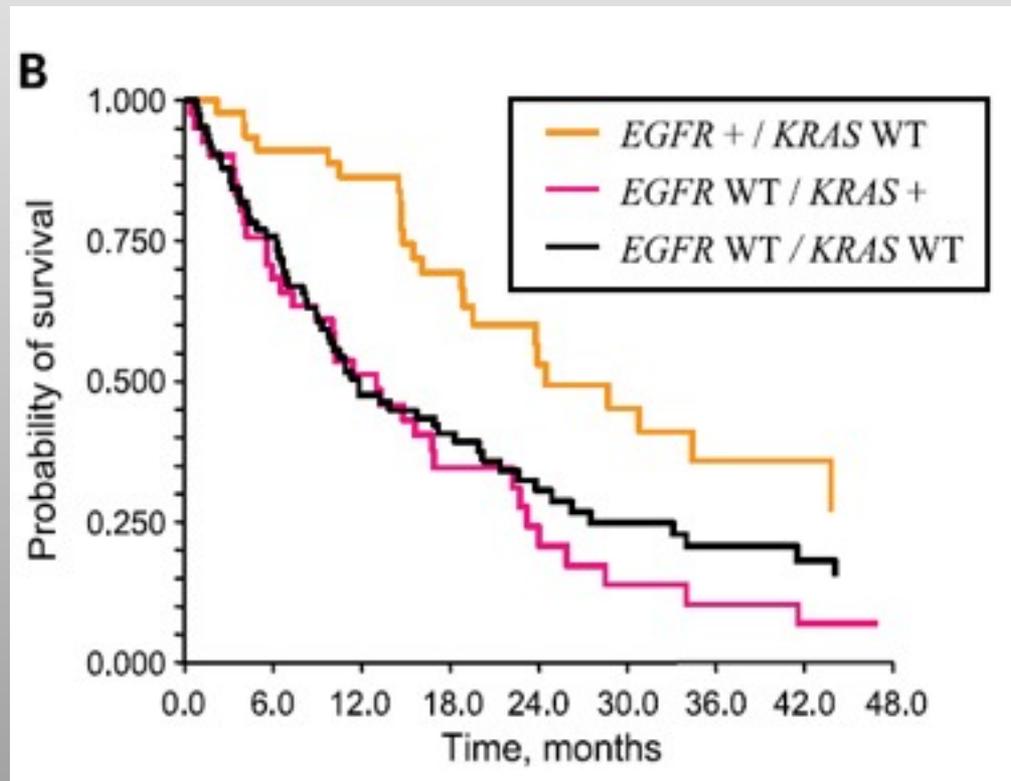
Nombre de copies



Sous-clones ?!

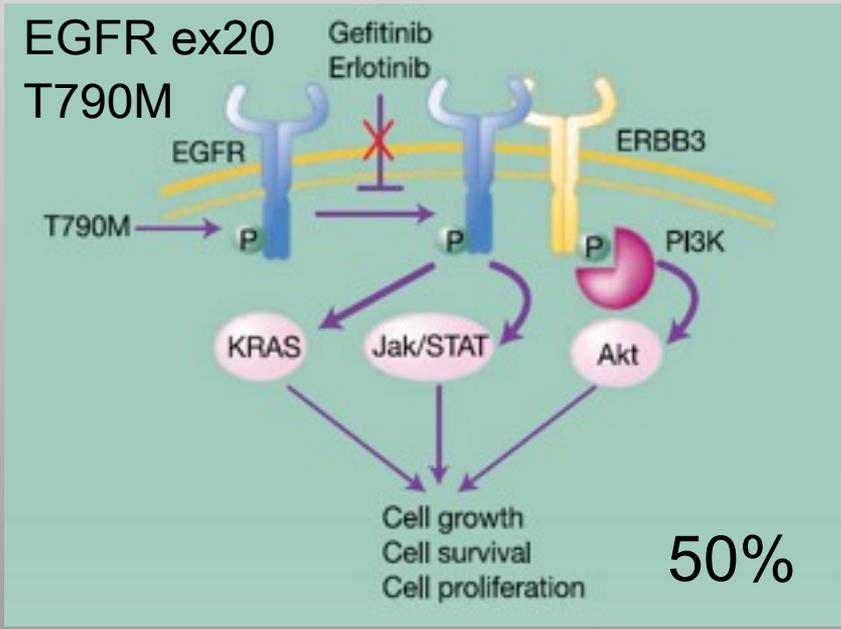


EGFR ou K-Ras ?

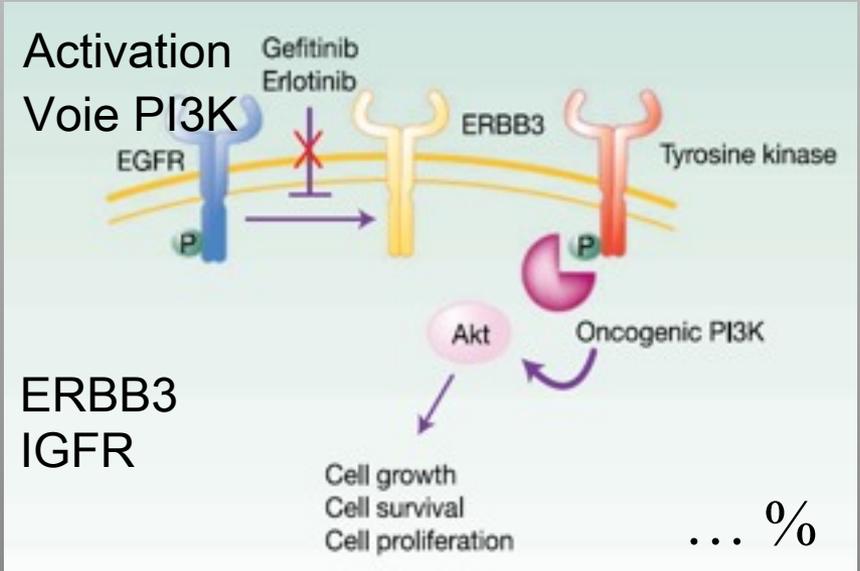
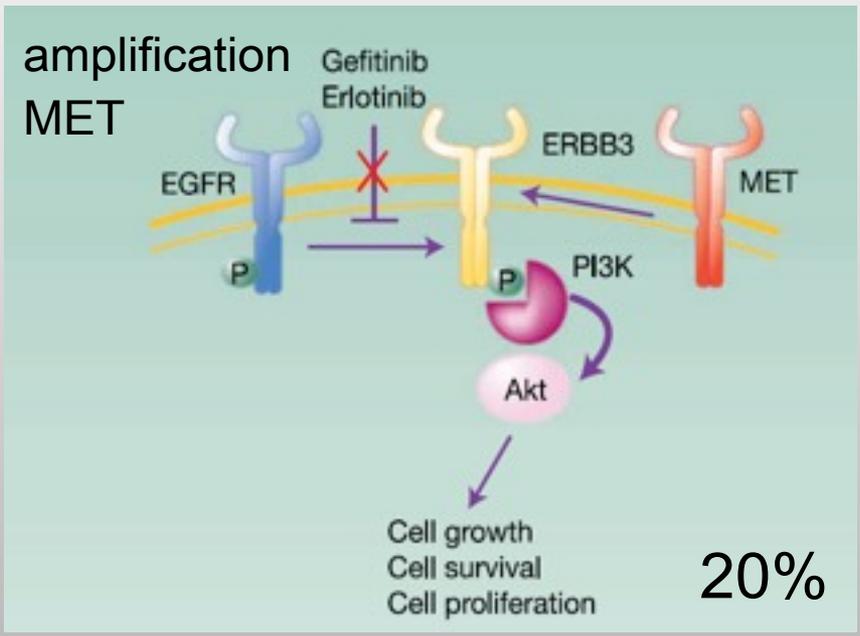


	<i>EGFR + / KRAS WT</i>	<i>EGFR WT / KRAS +</i>	<i>EGFR WT / KRAS WT</i>	P
N	47	41	83	
RR	68%	0	5%	< .001
Median TTP (months)	13.1	3.3	3.1	< .0001
Median OS	24.5	13.0	11.8	.002

Résistances secondaires

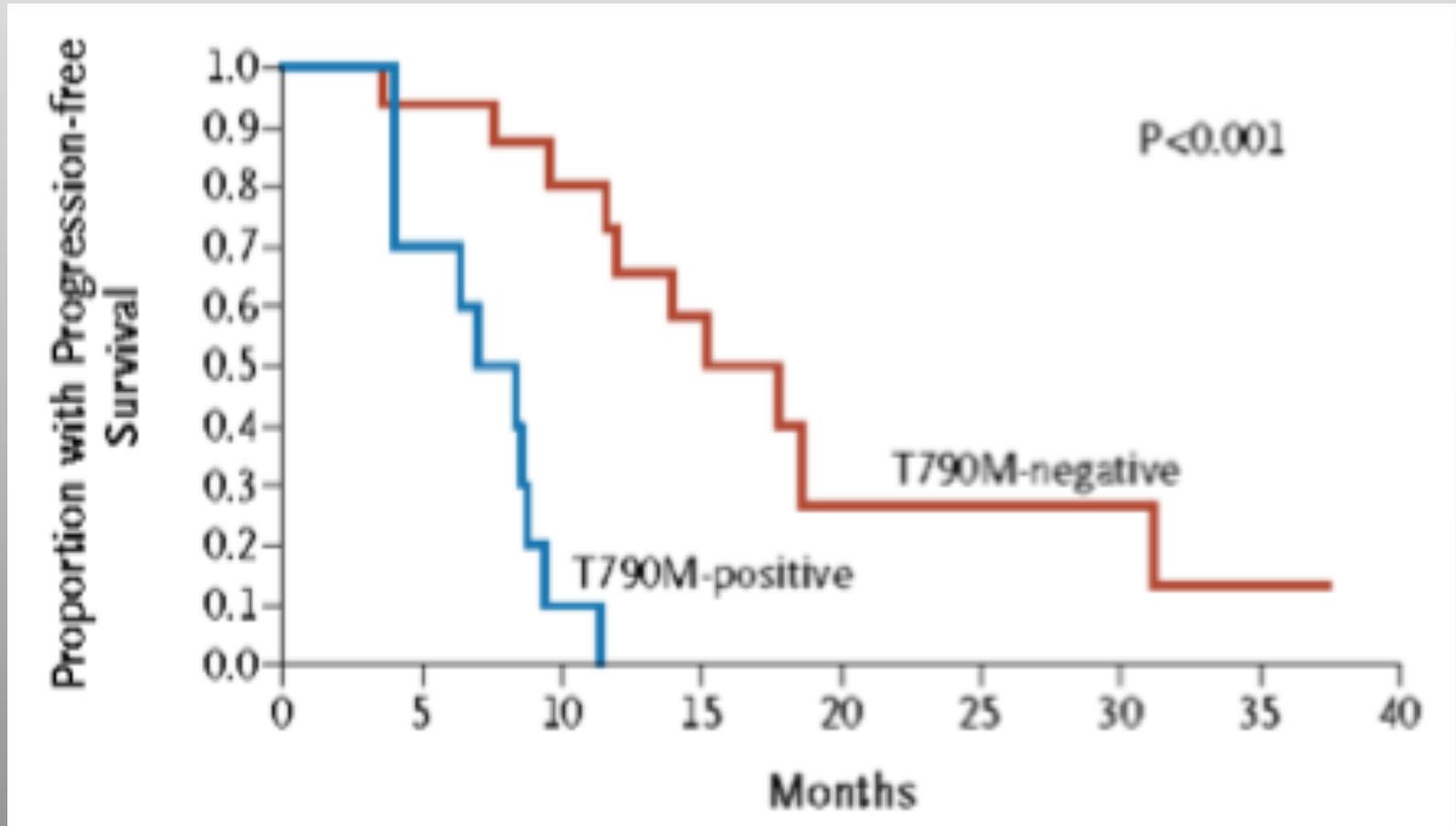


Hammerman PS CCR 2009



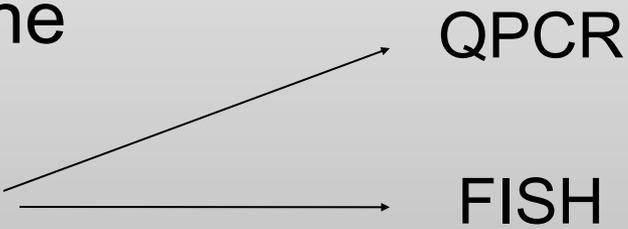
+ altération trafic EGFR

Mutation T790M et PFS



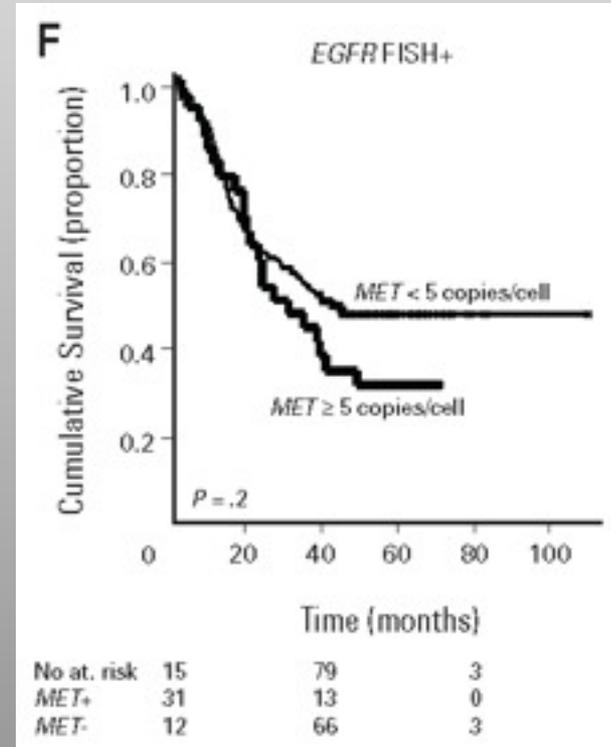
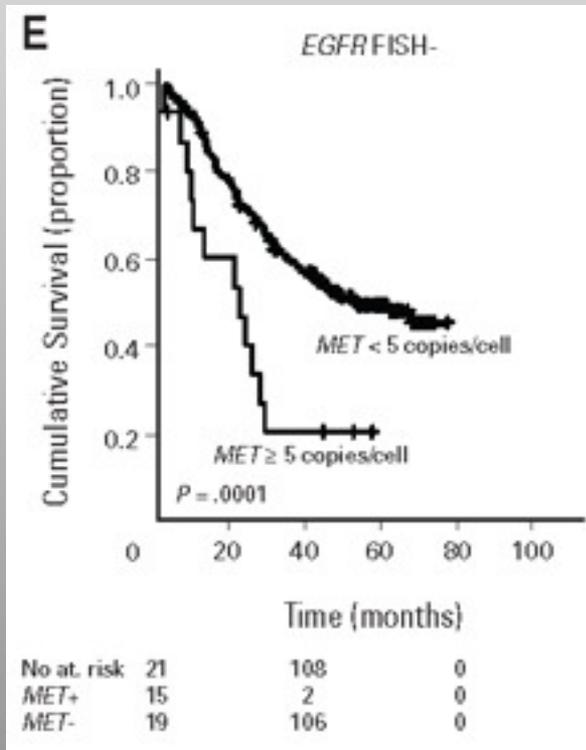
PFS: 7.7 mois vs 16.5 mois (95% CI: 2.94 - 45.1), $p > 0.001$

MET

- Proto-oncogène
 - Surexprimé
 - Amplification
 - dans 21% des tumeurs EGFR TKI résistantes
 - 3% tumeurs naïves d'EGFR TKI
 - Pas d'exclusivité avec les mutations d'EGFR
 - Marqueur de mauvais pronostic ?
 - Valeur prédictive ?
- 
- The diagram consists of two arrows originating from the text 'Surexprimé' and 'Amplification'. One arrow points from 'Surexprimé' to 'QPCR', and another arrow points from 'Amplification' to 'FISH'.

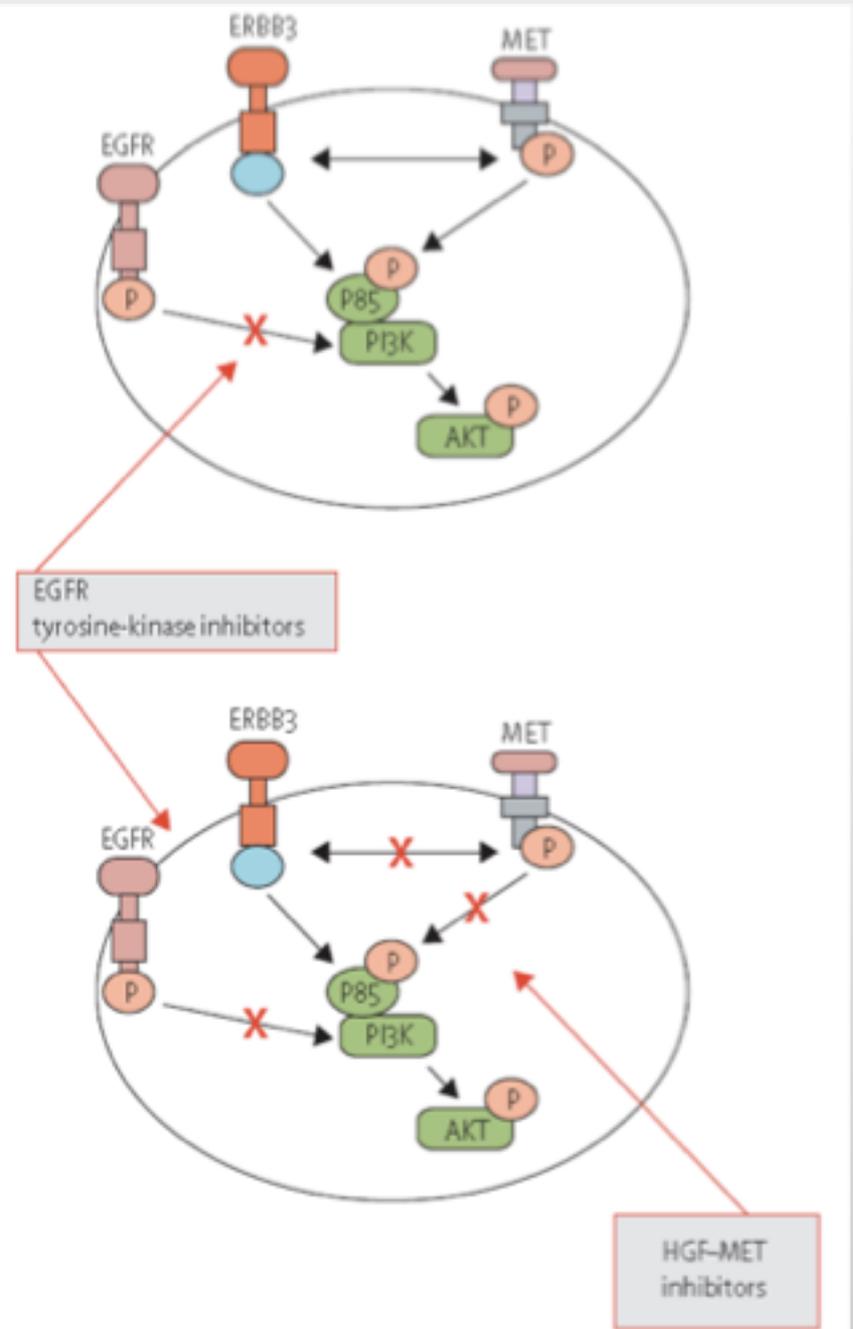
Amplification de MET

N = 447



Voie PI3K/AKT/mTOR

- Activation de la voie PI3K via ERBB3 ou MET
 - Étude pré-cliniques: inhibition de MET pourrait restaurer la sensibilité aux EGFR TKI
- > double inhibition



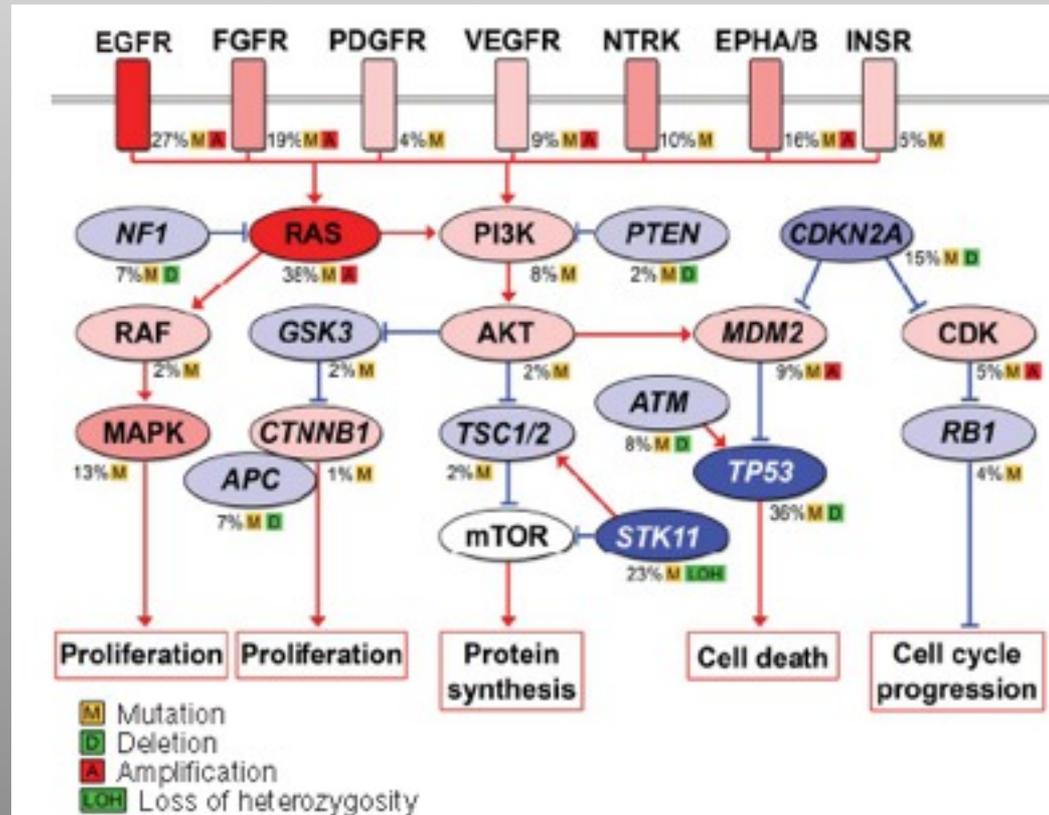
Les questions

- Surexpression/mutations d'autres acteurs de la voie EGFR

- HER2
- BRAF
- PI3K
- LKB1
- ...

- Mutations exclusives avec EGFR

- ... sauf PI3K

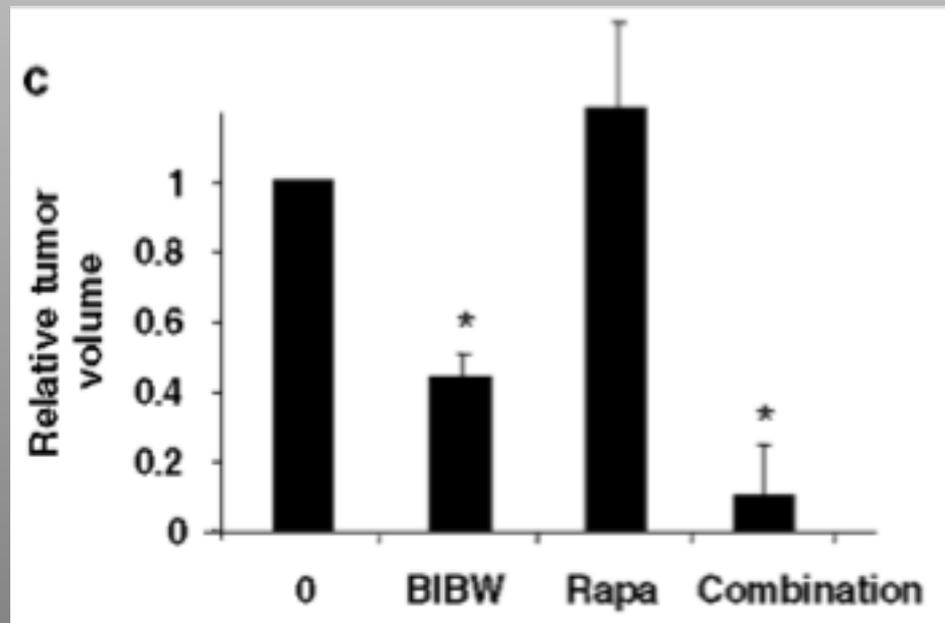
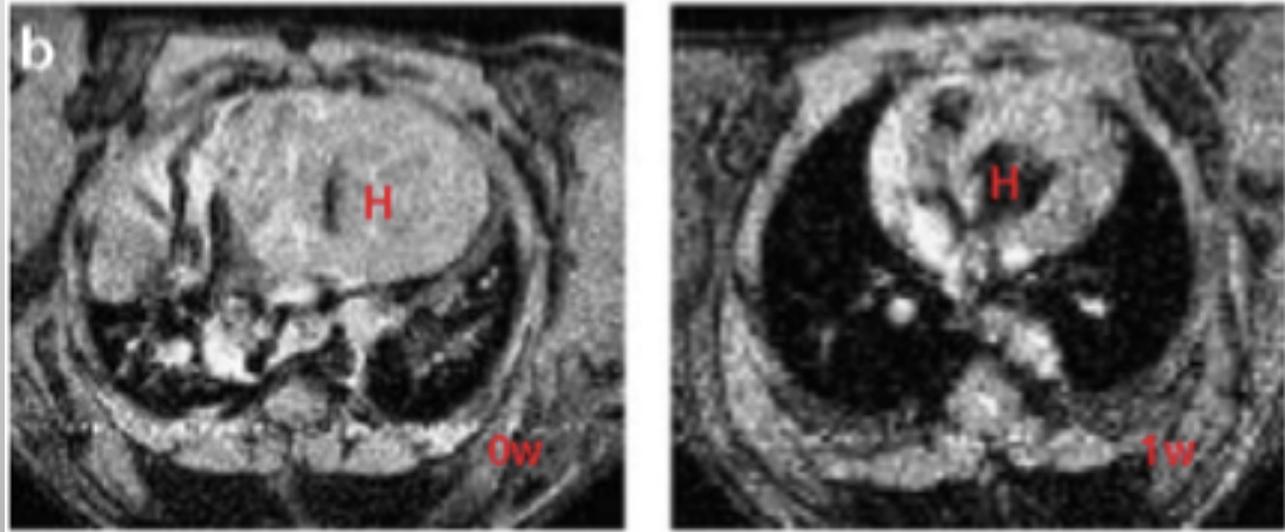


Que faire en cas de résistance ?

- Inhibiteurs irréversibles
 - Associations d'inhibiteurs
- BIBW2992
HKI-272 (neratinib)
- Inhibiteurs de la voie PI3K/mTOR

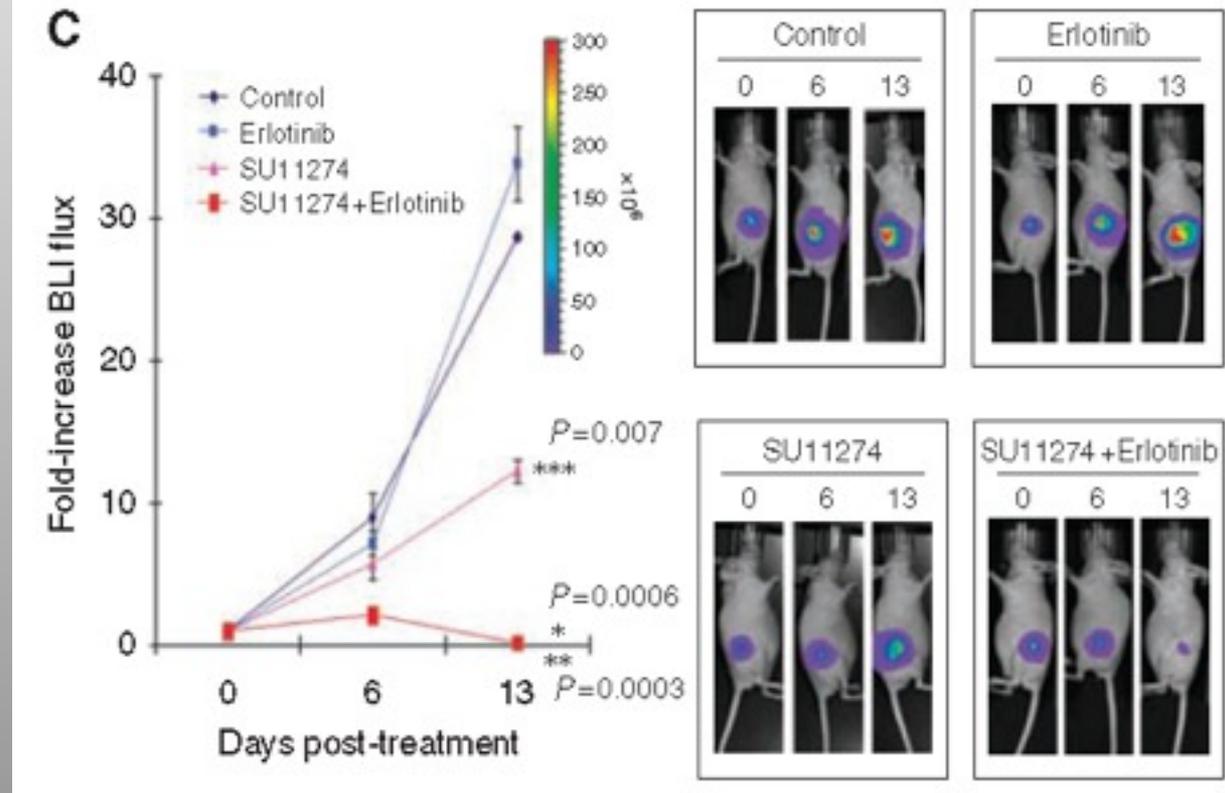
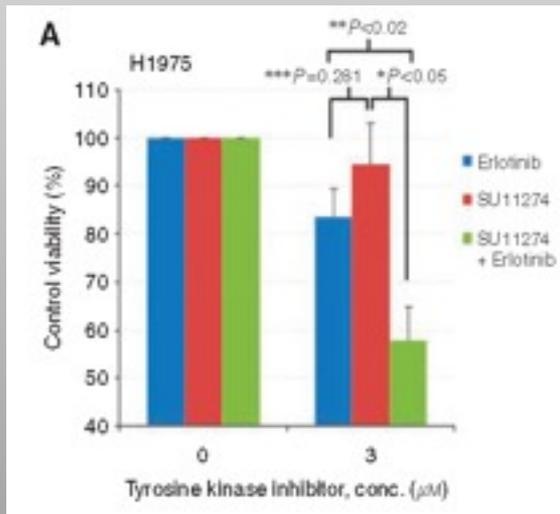
Combinaisons thérapeutiques: BIBW2992 et rapamycine

Modèle murin
L858R + T790M

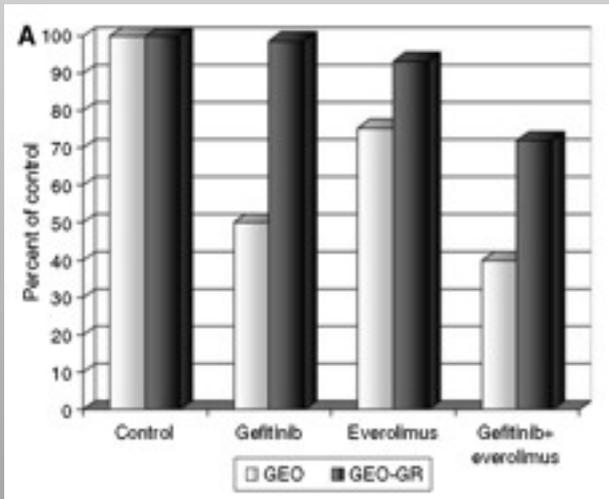


Combinaisons thérapeutiques: erlotinib et SU11274

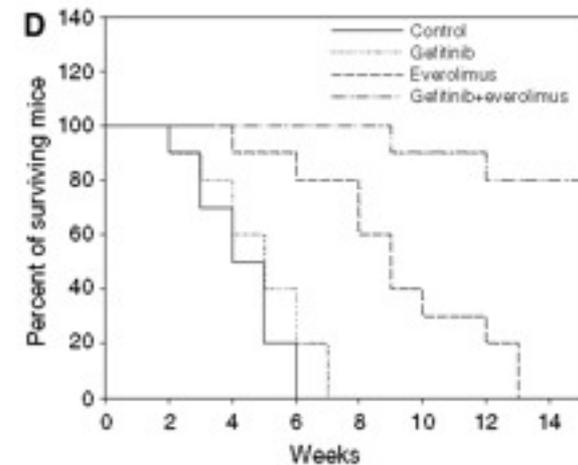
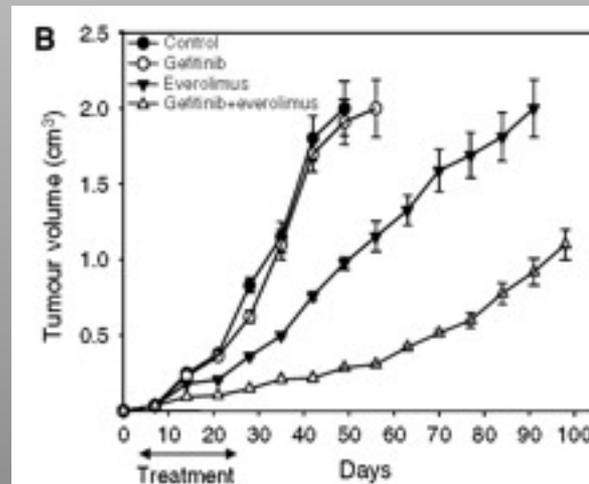
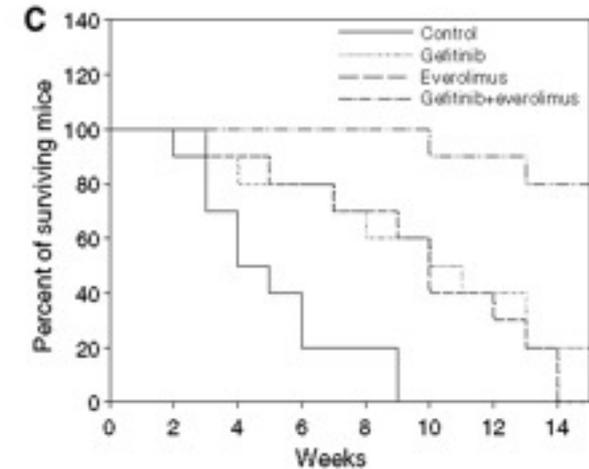
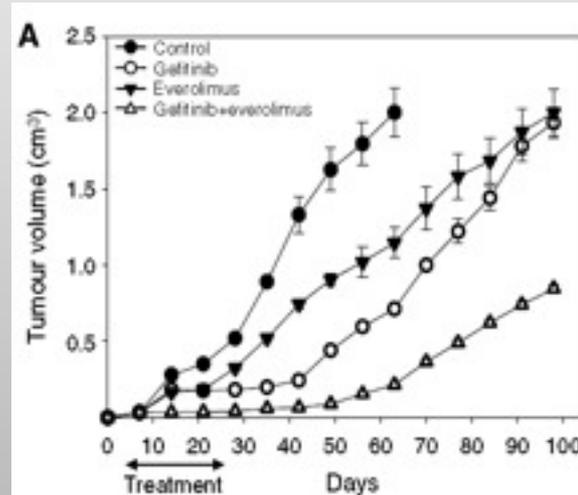
Modèle cellulaire
L858R+T790M (H1975)



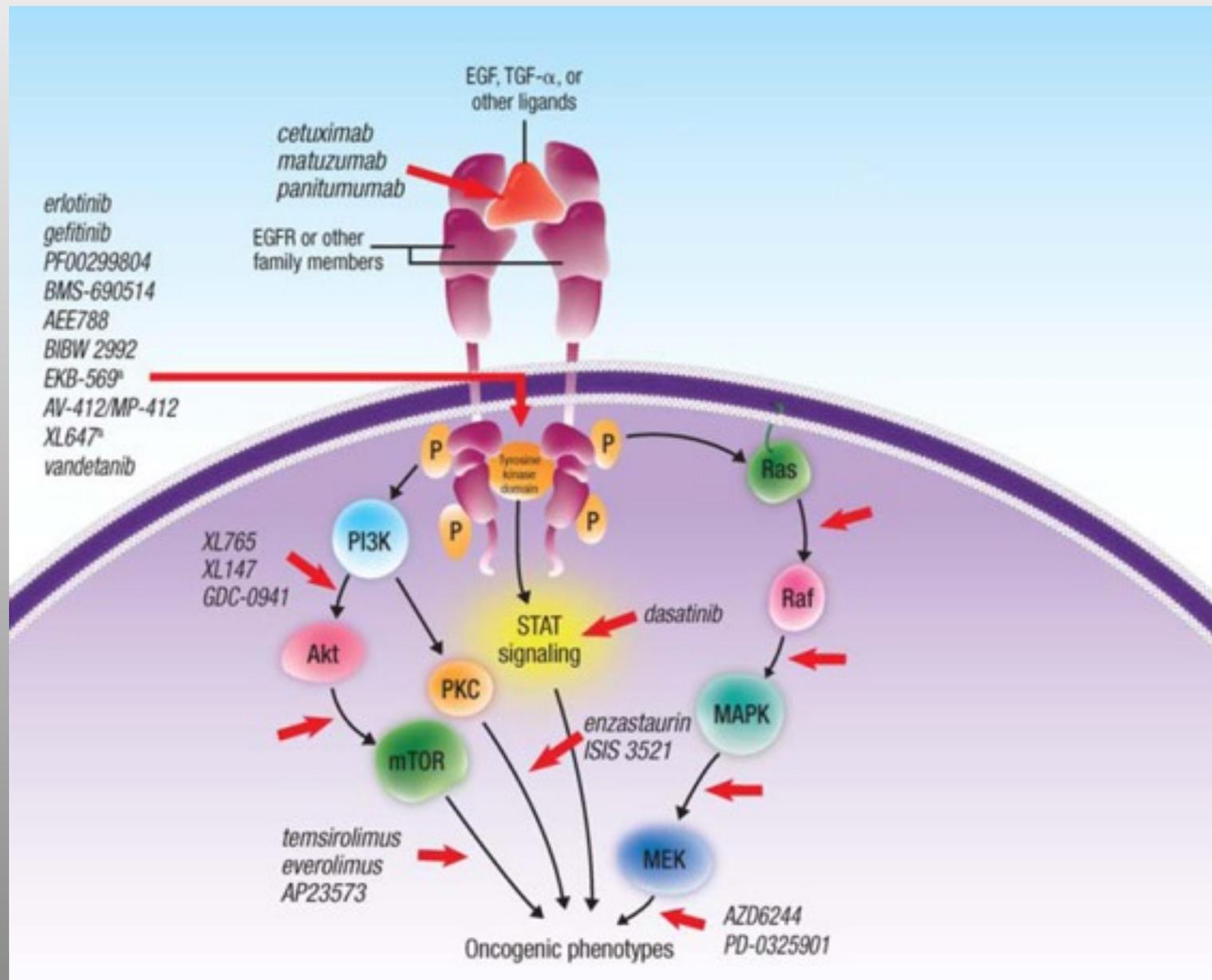
Combinaisons thérapeutiques: gefininib et rapamycine



In vitro: lignées



In vivo: xénogreffes



ERMETIC

Etude rétrospective

Validation de l'analyse par séquençage de la recherche des mutations d'EGFR et de K-Ras, partir de prélèvements de CBNPC inclus en paraffine, par 15 laboratoires français

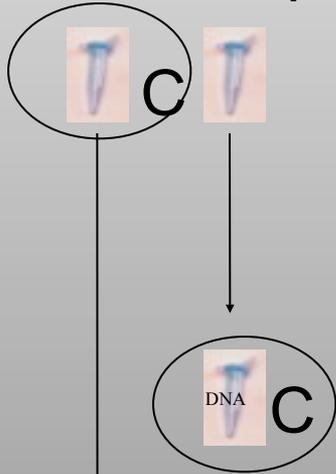
Dr. A. Degeorges, I. Curie

Dr. M. Beau-Faller, CHU Strasbourg

Schéma de l'étude

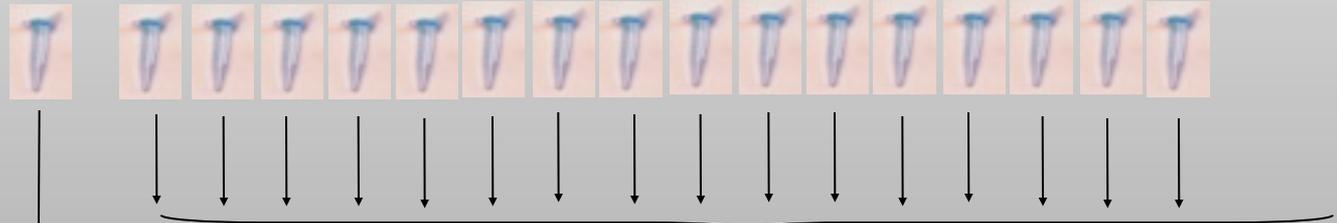
Congélation

2 x 3 coupes

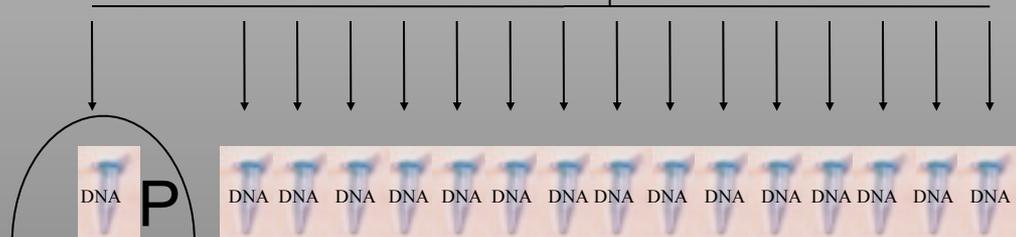


Paraffine

17 x 3 coupes



16 extractions d'ADN poolées



Centre moléculaire externe

15 centres ERMETIC

Méthodes

- Mutations d'EGFR: séquençage des exons 18 à 21
- Mutations de K-Ras: séquençage de l'exon 2

- Algorithme décisionnel
 - Échantillon non-amplifiable: après 3 échecs de PCR
 - Toute mutation est vérifiée par une 2ème PCR indépendante

- 15 centres: séquençage des ADN extraits de paraffine

- Centre externe:
 - séquençage des ADN extraits de congélation
 - séquençage des ADN extraits de paraffine

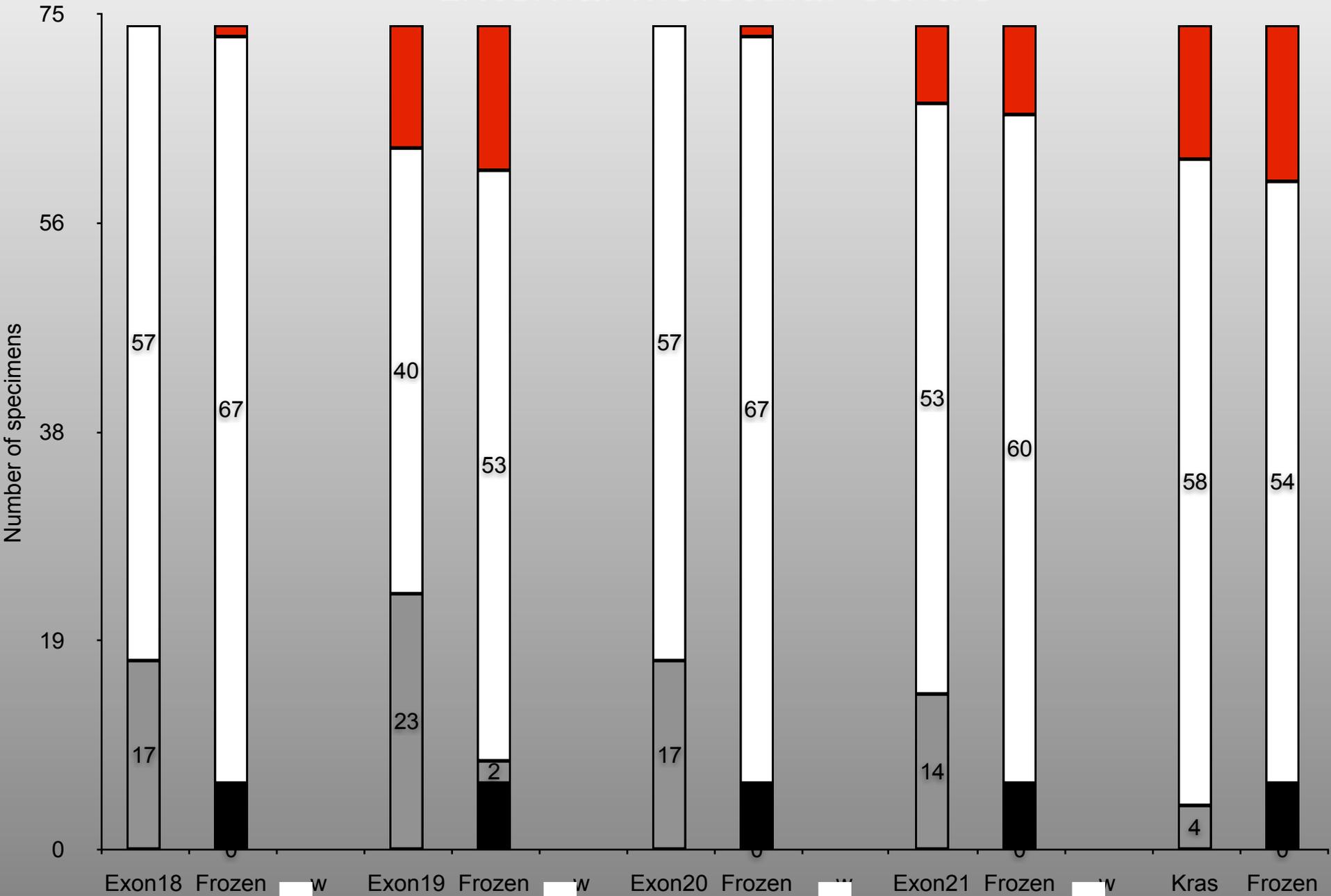
EGFR mutations

Frozen (n = 68)	Type of mutation	Paraffin (n = 68)
	<i>EGFR</i>	
	Exon 18 Mutations	
1	c.2156G>C, p.Gly719Ala) (G719A)*	NA
	Exon 19 Mutations	
5	c.2235_2249del ; p.Glu746_Ala750del **	5
2	c.2236_2250del ; p.Glu746_Ala750del	2
1	c.2239_2248delinsC ; p.Leu747_Glu749del,p.Ala750Pro	NA
1	c.2239_2251delinsC ; p.Leu747_Thr751delinsPro	1
1	c.2260A>G ; p.Lys754Glu (UV) **	1
1	c.2237_2251del ; p.Glu746_Thr751delinsAla	1
1	c.2239_2240TT>CC ; p.Leu747Pro (UV)	NA
1	c.2239_2256del ; p.Leu747_Ser752del	1
1	c.2240_2257del ; p.Leu747_Pro753delinsSer	1
	Exon 20 Mutations	
1	c.2311_2312insACCGGC ; p.Asp771_772insArg-His (UV)	NA
	Exon 21 Mutations	
7	c.2573T>G ; p.Leu858Arg (L858R)***	5+1WT +1NA
1	c.2543T>C ; p.Pro848Leu (UV)***	NA
1	c.2582T>A ; p.Leu861Gln (L861Q)*	1
25	Total EGFR	18

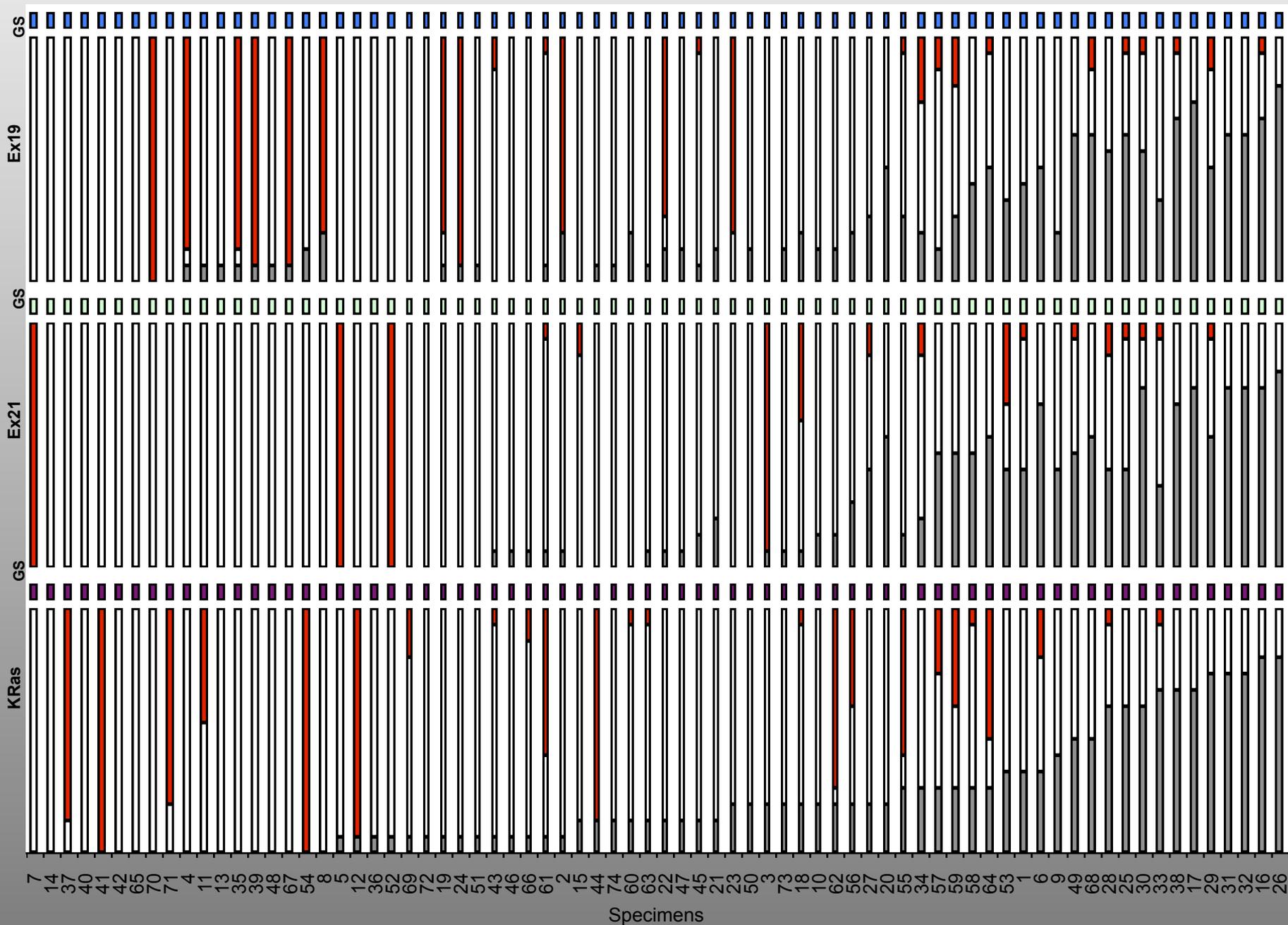
K-Ras mutations

Frozen (n= 68)	Type of mutation	Paraffin (n = 68)
<i>K-Ras</i>		
6	c.35G>A ; p.Gly012Asp (G12D)	5+1NA
6	c.34G>T ; p.Gly012Cys (G12C)	5+1WT
2	c.35G>T ; p.Gly012Val (G12V)	1+1WT
14	Total <i>K-Ras</i>	11

External molecular centre



Sequencing of blinded FFPE samples by the 15 ERMETIC centres



False positive/negative results by sample (n=74)

	<i>EGFR 19</i>	<i>EGFR 21</i>	<i>K-Ras</i>
Number of samples with at least one centre with a false positive result	10/74	10/74	7/74
Number of samples with at least one centre with false negative results	7/74	4/74	12/74

False positive/negative results by centre (n=15)

EGFR exon 19

EGFR exon 21

K-Ras

False positive results

Median rate of false positive results by centre, (Range)

0% (0-27%)

17% (0-44%)

8% (0-25%)

Number of centres with a false positive rate of:	0%	9/15	7/15	5/15
	> 0%, < 10%	1/15	0/15	4/15
	≥10%	5/15	8/15	6/15

False negative results

Median rate of false negative results by centre, (Range)

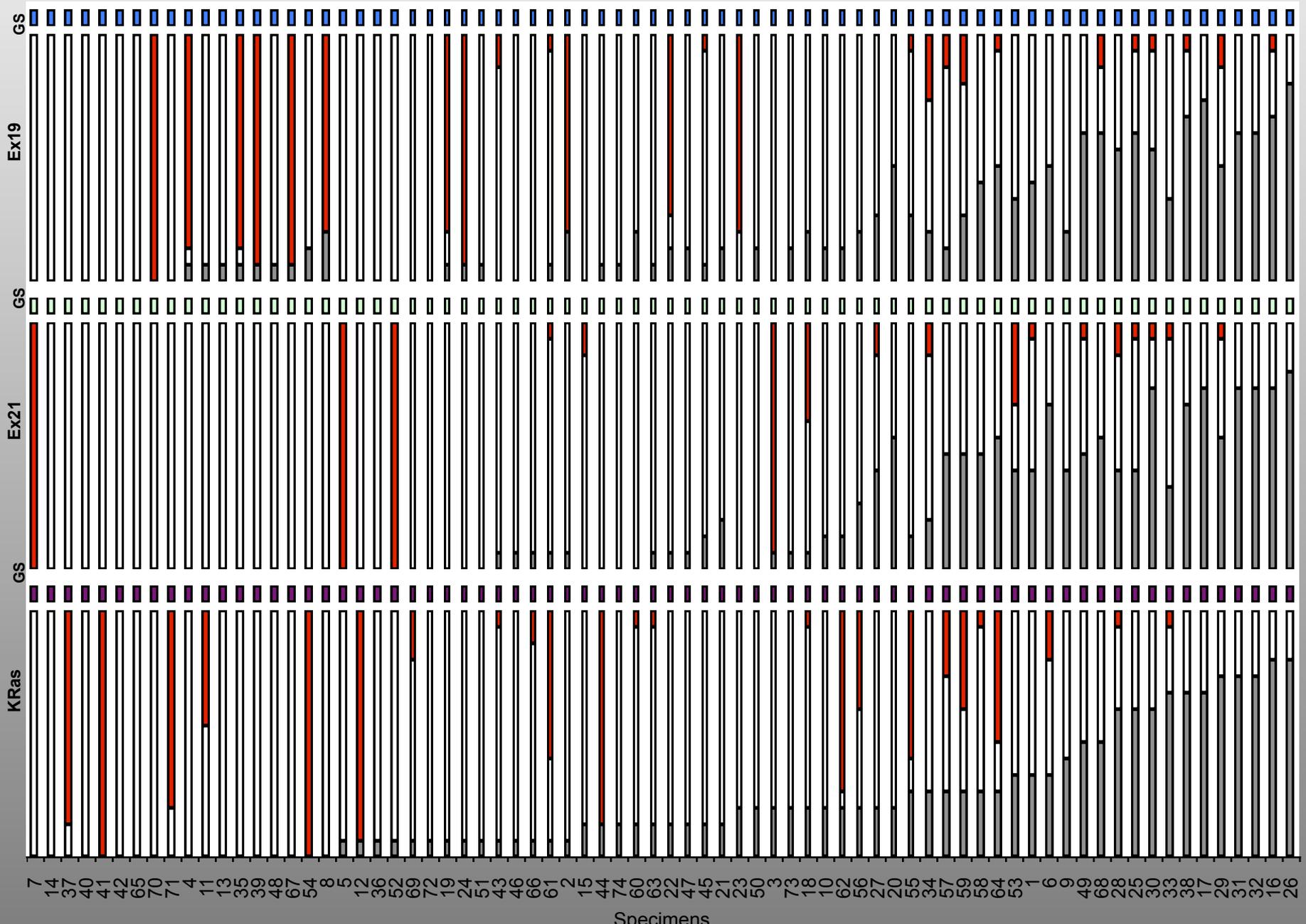
2% (0-9%)

3% (0-6%)

9% (2-17%)

Number of centres with a false negative rate of:	0%	6/15	6/15	0/15
	> 0%, < 10%	9/15	9/15	8/15
	≥ 10%	0/15	0/15	7/15

Non-amplifiable paraffin samples



Concordance of each ERMETIC centre with the Gold Standard

Kappa Score Rating	Number of centres with Kappa Score Rating		
	<i>EGFR 19</i>	<i>EGFR 21</i>	<i>K-Ras</i>
Good	9/15	0/15	3/15
Moderate	1/15	6/15	3/15
Poor	4/15	9/15	8/15
Bad	0/15	1/15	2/15
Median Kappa Score (Range)	0.52 (0.23-0.73)	0.37 (0.20-0.57)	0.39 (0.15-0.66)

Concordance between the 15 ERMETIC centres

Samples compared	Global Kappa Score (95% CI)		
	<i>EGFR 19</i>	<i>EGFR 21</i>	<i>K-Ras</i>
All samples (n=74)	0.47 (0.45-0.49)	0.47 (0.45-0.49)	0.42 (0.40-0.43)
Sensitivity analysis*(n=58)	0.60 (0.58-0.61)	0.56 (0.55-0.58)	0.47 (0.45-0.49)



Discussion

- **Phase A**: pas de mutation exotique ou de faux positifs; rares faux-négatifs (n= 3)
- **Phase B**:
 - Faux-positifs: varient selon l'exon et selon le centre; résultats validés par des techniques plus sensibles
 - Faux-négatifs: varient selon la qualité de l'échantillon et selon le centre
- **ADN Non-Amplifiable**: dépend des processus de fixation de l'âge et des conditions de stockage des blocs, des procédures d'extraction
- **Concordance « modérée » dans la détection des mutations**

Conclusion

- La recherche de mutations est possible sur de l'ADN extrait de blocs paraffine
- Des critères de qualités paraissent indispensables
 - Blocs âgés de moins de 5 ans
 - Tissus fixés en formaline
 - Échantillons contenant au moins 50% de cellules tumorale
- Utilisation de protocoles standardisés pour l'extraction d'ADN
- Questions à débattre:
 - Quelle signification clinique des faux-positifs et faux-négatifs?
 - Quel seuil de détection des mutations ?

Perspectives

ERMETIC-2

Apport des **techniques sensibles** de la recherche des mutations EGFR/K-Ras pour la prédiction de la sensibilité/résistances aux anti-EGFR TKI

- **recensement** des ADN/blocs restants: cohorte prospective et rétrospective
- **validation** des techniques alternatives sur cohorte **rétrospective**
- sélection de techniques alternatives à appliquer aux ADN de la cohorte **prospective**